

BD RiboQuant™ RNase Protection Assay Systems

BD Biosciences

Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen



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Introduction

Ribonuclease protection assay (RPA) is a highly sensitive and specific method to detect and quantitate RNAs. The method is based on the hybridization of a target RNA to either a ³²P- or biotin labeled anti-sense RNA probe *in vitro* transcribed from a DNA template. RNase treatment follows, resulting in degradation of single-stranded RNA and excess probe.

In the radioactive approach, the probe and target RNA are resolved by denaturing polyacrylamide gel electrophoresis and the “protected” probe is visualized using autoradiography or beta imaging equipment.

In our new non-radioactive approach, biotin-labeled probe is hybridized, in excess, to target RNA in solution. Following hybridization, free probe and other single-stranded RNA are digested with RNases. The remaining “RNase-protected” probes are purified, resolved on denaturing polyacrylamide gels, then transferred to a positively-

charged nylon membrane. The membrane is probed and the signal quantified using Streptavidin-HRP and the enhanced chemiluminescent substrate provided in the kit. The membrane is exposed to either X-ray film or a CCD camera and the level of each mRNA species in the original RNA sample is determined based on the intensity of the appropriately-sized, protected probe fragment.

The Multi-Probe RPA is made possible by generating a series of templates, each of a different length and each representing a sequence in a distinct mRNA species. The templates are assembled into biologically relevant sets and used for the T7 polymerase-directed synthesis of a high-specificity, biotin-labeled, anti-sense RNA probe set.

Each template set is capable of detecting up to 12 unique gene messages in addition to two housekeeping genes, L32 and GAPDH.

The multi-probe RPA approach has the distinct advantages of sensitivity and the capacity to simultaneously quantify several mRNA species in a single sample of total RNA. This allows comparative analyses of different mRNA species within samples and, by incorporating a probe for a housekeeping gene transcript, the levels of individual mRNA species can be compared between samples. Moreover, the assay is highly specific and quantitative due to the RNase sensitivity of mismatched base pairs and the use of solution-phase hybridization driven toward completion by excess probe. Lastly, the Multi-Probe RPA can be performed on total RNA preparations derived by standard methods from either cultured cells or frozen tissues, without further purification of poly-A⁺ RNA.

BD RiboQuant™ RNase Protection Assay System Advantages:

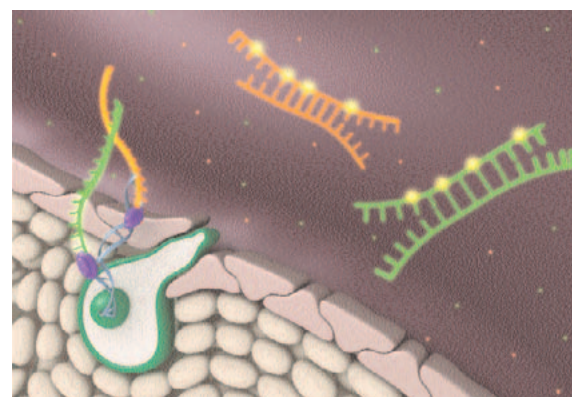
- Easy, multiplexed quantification of mRNA
- Uses non-radioactive biotin-labeled probes
- Highly specific
 - High-stringency hybridizations
 - Comparisons within and between mRNA species
- Highly sensitive:
 - Solution-phase hybridization at probe excess
 - Stability of RNA hybrids
 - Linear signal over broad input RNA range
- Uses total RNA from cells or tissues

Unparalleled range of specificities available

We offer over 100 pre-assembled, biologically relevant Multi-Probe Template Sets and over 1000 specific templates from which to choose.

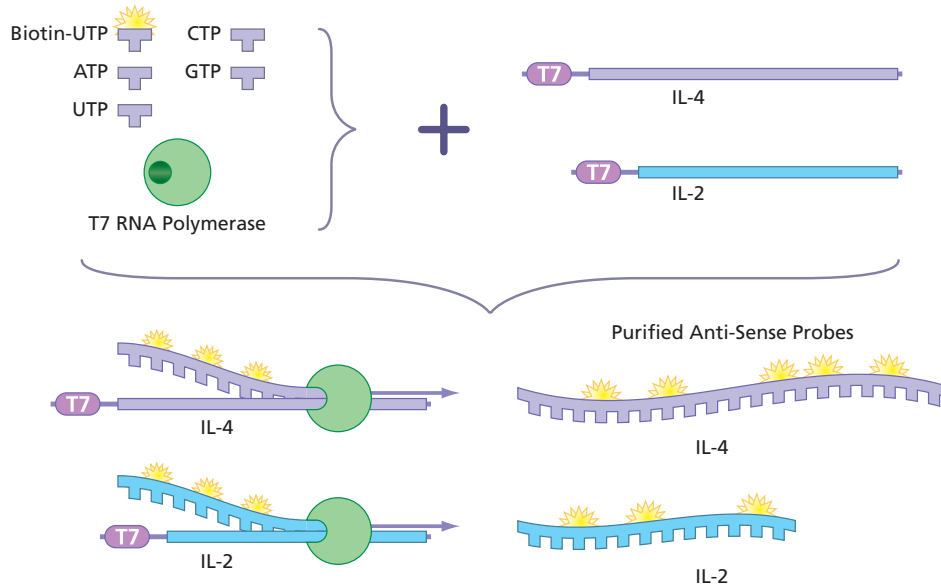
Custom Template Assembly and Design

We also offer custom template construction and/or assembly to meet your needs.

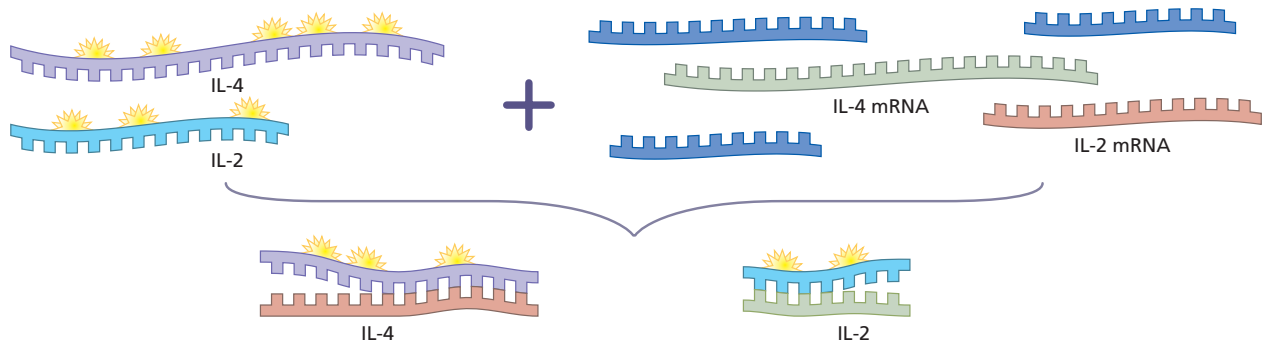


Overview of the BD RiboQuant™ Procedure

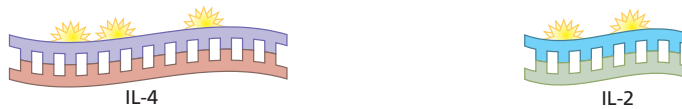
In Vitro Transcription



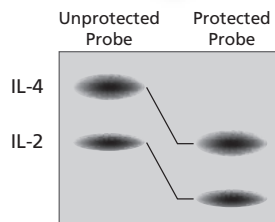
Hybridization



RNase Digestion



Resolve on Denaturing Gel. Transfer to Membrane.*



* Transfer to Membrane applies to Non-Rad RPA Procedure ONLY.

Non-Radioactive RNase Protection Assay for Multiplexed Quantification of Differentially Expressed Genes

The new version of the BD RiboQuant™ Ribonuclease Protection Assay (RPA) offers a non-radiolabeled (Non-Rad) approach to this powerful and versatile system. Multi-Probe RPA has traditionally been performed using probes labeled by the incorporation of [³²P]-UTP to provide optimal signal and sensitivity. By using our new kit to generate non-radiolabeled probes for Multi-Probe RPA, risks associated with exposure to radioactivity, environmental concerns and regulatory requirements are eliminated. With our non-radioactive kits, researchers can now choose which system best suits their needs.

The BD RiboQuant™ Non-Rad RPA System includes:

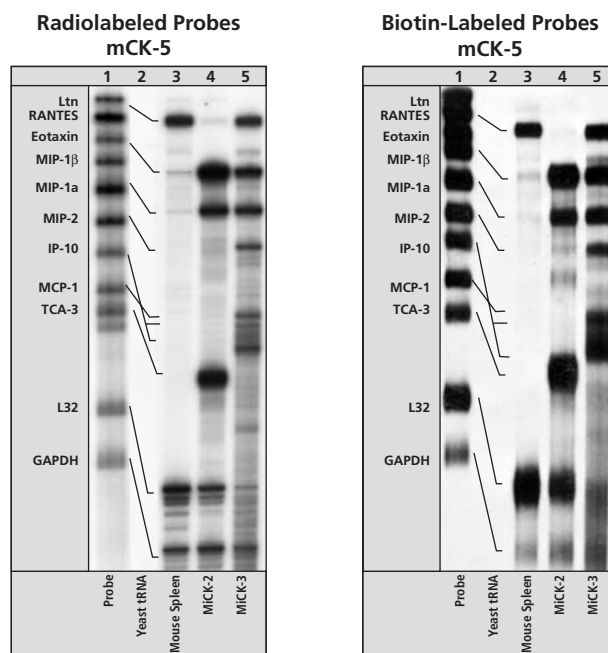
- Multi-Probe Template Sets
- BD RiboQuant Non-Rad In Vitro Transcription Kit* (Cat. No. 551917)
- BD RiboQuant RPA Kit (Cat. No. 556134)
- BD RiboQuant Non-Rad Detection Kit (Cat. No. 551918)
- BD RiboQuant Non-Rad Starter Package† (Cat. No. 551919)

BD RiboQuant Non-Rad In Vitro Transcription Kit

The BD RiboQuant Non-Rad *In Vitro* Transcription Kit is optimized for the efficient synthesis of high specific-activity, biotin-labeled riboprobes from the BD Pharmingen™ Multi-Probe Template Sets. Each kit contains sufficient reagents for 5 transcription reactions, yielding ~10 µg biotin-labeled probe per reaction.

BD RiboQuant Non-Rad Detection Kit

The BD RiboQuant Non-Rad Detection Kit includes 10 positively-charged nylon membranes and all reagents necessary for signal development using enhanced chemiluminescence.



mCK-5
Cat. No. 556146

Template	Probe	Protected
LtN	390	361
RANTES	349	321
Eotaxin	314	285
MIP-1β	285	257
MIP-1α	256	228
MIP-2	231	205
IP-10	210	181
MCP-1	190	163
TCA-3	171	143
L32	141	112
GAPDH	125	97

Non-Rad vs. Radiolabeled RPA using mCK-5.

RPA was performed using 10 µg total RNA from various cell populations. Lane 3) untreated mouse spleen. Lane 4) Purified CD4⁺ splenocytes (BALB/c) were stimulated with anti-mouse CD3 and anti-mouse CD28, and cultured with purified mouse IL-2 and mouse IL-4, followed by restimulation with PMA + ionomycin (MiCK-2). Lane 5) 3-day thioglycolate-elicited peritoneal cells (BALB/c) stimulated with purified mouse IFN-γ and LPS in the presence of a protein transport inhibitor (MiCK-3). (For complete stimulation protocols, please refer to Cytokines/Chemokines Application Manual, 3rd ed., BD Biosciences Pharmingen, June 1999, pp 31-32.)

* Biotinylated nucleotides must be purchased separately. BD RiboQuant non-radioactive RPA has been optimized with biotin-16-UTP available from Roche Biosciences.

† Individual kits or sets of the system may be purchased separately or together, with one Multi-Probe Template Set of choice, as the BD RiboQuant Non-Rad RPA Starter Package, Cat. No. 551919. All protocols are described in the newest edition of the BD RiboQuant Instruction Manual. Please contact your local BD Biosciences office.

BD RiboQuant™ Reagents and Kits

556850 BD RiboQuant™ *In Vitro* Transcription Kit

Kit Contents

556133	Ammonium Acetate	1.3 ml
556126	DTT	50 µl
556132	EDTA	650 µl
556125	GTP/ATP/CTP/UTP Pool	25 µl
556130	RNase-Free DNase	50 µl
556128	RNasin®*	25 µl
556129	T7 RNA Polymerase	25 µl
556127	Transcription Buffer	100 µl
556131	Yeast tRNA	50 µl

551917 BD RiboQuant™ Non-Rad *In Vitro* Transcription Kit

Kit Contents

	5x Nucleotide Mix	16.5 µl
	5x Transcription Buffer	20 µl
	DTT	10 µl
	Enzyme Mix (RNasin®* & T7 RNA Polymerase)	10 µl
	RNase-free DNase I	10 µl
	EDTA	135 µl
	Glycogen	5 µl
	LiCl	2.7ml

556134 RPA Kit

Kit Contents

556141	Ammonium Acetate	24 ml
556135	Hybridization Buffer	3.6 ml
556142	Loading Buffer	1.3 ml
556139	Proteinase K	300 µl
556138	Proteinase K Buffer	3.9 ml
556137	RNase A + T1 Mix	60 µl
556136	RNase Buffer	25 ml
556140	Yeast tRNA	300 µl

551918 BD RiboQuant™ Non-Rad Detection Kit

Kit Contents

	Nylon Membranes	10
	Hybridization Buffer	100 ml
	Hybridization Stringency Wash Buffer	300 ml
	Membrane Blocking Buffer	400 ml
	Wash Buffer (4x)	400 ml
	Substrate Equilibration Buffer	400 ml
	Streptavidin-Horseradish Peroxidase	1.5 ml
	Stable Peroxide Buffer	50 ml
	Luminol / Enhancer	50 ml

556144 BD RiboQuant™ RPA Starter Package

Package Contents

556850	In Vitro Transcription Kit	1 Kit
556134	RPA Kit	1 Kit
	Choice of one BD RiboQuant™ Template Set	10 reactions

551919 BD RiboQuant™ Non-Rad RPA Starter Package

Package Contents

551917	BD RiboQuant™ Non-Rad In Vitro Transcription Kit	1 Kit
556134	RPA Kit	1 Kit
551918	BD RiboQuant™ Non-Rad Detection Kit	1 Kit
	Choice of one BD RiboQuant™ Template Set	10 reactions

556224 Total RNA Isolation Kit

Composition of Available Template Sets

Human Cytokines/Chemokines

Template Set	hCK-1	hCK-2b	hCK-3	hCK-4	hCK-5	hCK-8	hCK-9
Catalog Number	556151	559242	556153	556154	556155	551787	551488
Templates	IL-5	IL-12p35	TNF-β	IL-3	Ltn	Eotaxin	TECK
	IL-4	IL-12p40	LTβ	IL-7	RANTES	RANTES	MPIF-1
	IL-10	IL-10	TNF-α	GM-CSF	IP-10	MCP-4	MDC
	IL-14	IL-1α	IFN-γ	M-CSF	MIP-1β	MIP-1β	SLC
	IL-15	IL-1β	IFN-β	G-CSF	MIP-1α	MCP-2	MIP-3α
	IL-9	IL-1Ra	TGFβ3	IL-6	MCP-1	MIP-1α	MIP-3β
	IL-2	IL-6	TGFβ2	LIF	IL-8	MCP-1	PARC
	IL-13	IGIF	TGFβ1	SCF	I-309	MCP-3	TARC
	IFN-γ	IFN-γ	L32	OSM	L32	I-309	HCC-4
	L32	L32	GAPDH	L32	GAPDH	HCC-1	HCC-2
	GAPDH	GAPDH		GAPDH		L32	L32
						GAPDH	GAPDH

* RNasin® is a registered trademark of Promega Corporation.

Composition of Available Template Sets *(continued)*

Mouse Cytokines/Chemokines

Template Set	mCK-1	mCK-1b	mCK-2b	mCK-3b	mCK-4	mCK-5c
Catalog Number	556121	556157	556156	556158	556145	551943
Templates	IL-4	IL-4	IL-12p35	TNF-β	IL-3	Ltn
	IL-5	IL-5	IL-12p40	LT-β	IL-11	RANTES
	IL-10	IL-10	IL-10	TNF-α	IL-7	MIP-1β
	IL-13	IL-13	IL-1α	IL-6	GM-CSF	MIP-1α
	IL-15	IL-15	IL-1β	IFN-γ	M-CSF	MIP-2
	IL-9	IL-9	IL-1Ra	IFN-β	G-CSF	IP-10
	IL-2	IL-2	IL-18/IGIF	TGF-β1	LIF	MCP-1
	IL-6	IL-3	IL-6	TGF-β2	IL-6	TCA-3
	IFN-γ	IFN-γ	IFN-γ	TGF-β3	SCF	Eotaxin
	L32	L32	MIF	MIF	L32	L32
	GAPDH	GAPDH	L32	L32	GAPDH	GAPDH
			GAPDH	GAPDH		

Rat and Pig Cytokines/Chemokines

Template Set	rCK-1	rCK-2	rCK-3	pCK-1	pCK-2	pCK-3	pCK-4
Catalog Number	556147	556244	556245	557062	557063	557350	557468
Templates	IL-1α	IL-1α	IFN-β	IL-4	IL-12p35	TNF-β	AMCF-11
	IL-1β	IL-1β	TNF-β	IL-10	IL-12p40	TNF-α	GM-CSF
	TNF-β	IL-1Ra	GM-CSF	IL-15	IL-10	IFN-β	G-CSF
	IL-3	IL-12p35	TGF-β1	IL-2	IL-1α	TGF-β2	MCP-1
	IL-4	IL-12p40	TGF-β3	IL-6	IL-1β	IL-6	MCP-2
	IL-5	IL-18/IGIF	TGF-β2	IFN-γ	IL-18	IFN-γ	IL-8
	IL-6	IL-6	LTβ	L32	IL-6	L32	RANTES
	IL-10	IL-10	TNFα	GAPDH	IFN-γ	GAPDH	L32
	TNF-α	MIF	MIF		L32		GAPDH
	IL-2	IFN-γ	IFN-γ		GAPDH		
	IFN-γ	L32	L32				
	L32	GAPDH	GAPDH				
	GAPDH						

Human and Mouse Cytokine Receptors

Template Set	hCR-1b	hCR-2	hCR-3b	hCR-4	hCR-5	hCR-6	hCR-7	hCR-8
Catalog Number	557197	556203	558833	556205	556206	556207	556208	556210
Templates	IL-13Rα1	IL-5Rα	IL-10R2	IL-1RI	CCR1	CXCR1	CCR5	STRL33
	IL-7Rα	IL-3Rα	IL-10R	IL-1RII	CCR3	CXCR2	L32	US28
	IL-9Rα	GM-CSFRα	IL-11R	TNFRp75	CCR4	CXCR3	GAPDH	CCR3
	IL-13Rα	βc	IL-12Rβ1	TNFRp55	CCR5	CXCR4		CCR5
	IL-15Rα	IL-6Rα	IL-12Rβ2	IL-6Rα	CCR8	BLR-1		CXCR4
	IL-4Rα	gp130	IL-6Rα	gp130	CCR2a+b	CCR7		CCR8
	γc	LIFRα	gp130	TGF-βRI	CCR2a	V28		GPR15
	IL-2Rβ	G-CSFR	IFN-γRα	TGF-βRII	CCR2b	L32		GPR1
	IL-2Rα	L32	IFN-γRβ	L32	L32	GAPDH		V28
	L32	GAPDH	L32	GAPDH	GAPDH			CCR2b
	GAPDH		GAPDH					L32
								GAPDH

Template Set	mCR-1	mCR-2	mCR-3	mCR-4	mCR-5	mCR-6
Catalog Number	556196	556197	556198	556199	556200	556201
Templates	IL-7Rα	IL-5Rα	IL-10R	IL-1RI	CCR1	CXCR2
	IL-9Rα	AIC2A	IL-11R	IL-1RII	CCR1b	CXCR4
	IL-13Rα	GM-CSFRα	IL-12Rβ1	TNFRp75	CCR3	BLR-1
	IL-15Rα	IL-3Rα	IL-12Rβ2	TNFRp55	CCR4	L32
	IL-4Rα	IL-6Rα	IL-6Rα	IL-6Rα	CCR5	GAPDH
	γc	gp130	gp130	gp130	CCR2	
	IL-2Rβ	AIC2B	IFN-γRα	TGF-βRI	L32	
	IL-2Rα	LIFRα	IFN-γRβ	TGF-βRII	GAPDH	
	L32	G-CSFR	L32	L32		
	GAPDH	L32	GAPDH	GAPDH		
		GAPDH				

Composition of Available Template Sets *(continued)*

Human and Mouse Cell Surface Antigens

Template Set	hCD-1	mCD-1
Catalog Number	556230	556228
Templates	TCR δ	TCR δ
	TCR α	TCR α
	CD3 ϵ	CD3 ϵ
	CD4	CD4
	CD8 α	CD8 α
	CD8 β	CD8 β
	CD19	CD19
	CD14	F4/80
	CD45	CD45
	L32	L32
	GAPDH	GAPDH

Human Apoptosis

Template Set	hAPO-1b	hAPO-1c	hAPO-2b	hAPO-2c	hAPO-3	hAPO-3b
Catalog Number	556209	556233	556240	556235	556163	556237
Templates	Caspase-8	Caspase-8	bcl-w	bcl-w	Caspase-8	Caspase-8
	Granzyme B	Caspase-4	bcl-x (L)	bcl-x (L)	FasL	FasL
	Caspase-3	Caspase-3	bcl-x (S)	bcl-x (S)	Fas	Fas
	Caspase-6	Caspase-6	bfl-1	bfl-1	FADD	CLARP
	Caspase-5	Caspase-10a	BID	bad	DR3	FAP
	Caspase-2 (S)	Caspase-5	bik	bik	FAP	CRADD
	Caspase-7	Caspase-2 (S)	bak	bak	FAF	DAXX
	Caspase-1	Caspase-7	bax	bax	TRAIL	MADD
	Caspase-2 (L)	Caspase-1	bcl-2	bcl-2	TNFRp55	RIP
	Caspase-9	Caspase-2 (L)	mcl-1	mcl-1	TRADD	L32
	L32	Caspase-9	L32	L32	RIP	GAPDH
	GAPDH	L32	GAPDH	GAPDH	L32	GAPDH
		GAPDH			GAPDH	

Template Set	hAPO-3d	hAPO-4	hAPO-5	hAPO-5b	hAPO-5c	hAPO-6
Catalog Number	557278	556164	556165	556236	556239	556238
Templates	Caspase-8	Granzyme A	XIAP	TRAF1	XIAP	IPL
	FasL	Granzyme B	TRAF1	TRAF2	Survivin	ASK1
	Fas	DAD1	TRAF2	TRAF4	NAIP	Harakiri
	DcR-1	FAST K	TRAF4	I-TRAF	c-IAP-2	SIAH
	DR3	Granzyme H	NAIP	TRAF5	c-IAP-1	DFF
	DR5	RVP1	c-IAP-2	TRAF6	TRPM-2	Nip2
	DR4	Dr-nm23	c-IAP-1	TRAF3	L32	Nip3
	TRAIL	Granzyme 3	TRPM-2	TRIP	GAPDH	Nip1
	DcR-2	Requiem	TRAF3	L32		DAP-K
	TNFRp55	CAS	L32	GAPDH		DAP
	TRADD	Perforin	GAPDH			DRM
	RIP	L32				L32
	L32	GAPDH				GAPDH
	GAPDH					

Mouse and Rat Apoptosis

Template Set	mAPO-1	mAPO-2	mAPO-3	rAPO-1
Catalog Number	556195	556191	556192	556227
Templates	caspase-8	bcl-w	caspase-8	Fas
	caspase-3	bfl1	FasL	bcl-x (L)
	caspase-6	bcl-x (L)	Fas	bcl-x (S)
	caspase-11	bcl-x (S)	FADD	FasL
	caspase-12	bak	FAP	caspase-1
	caspase-2 (L)	bax	FAF	caspase-3
	caspase-7	bcl2	TRAIL	caspase-2
	caspase-1	bad	TNFRp55	bax
	caspase-14	L32	TRADD	bcl-2
	caspase-2 (S)	GAPDH	RIP	L32
	L32		L32	GAPDH
	GAPDH		GAPDH	

Human Cell Cycle Regulators

Template Set	hCC-1	hCC-2	hCYC-1	hCYC-2	hStress-1	hTS-1
Catalog Number	556159	556160	556189	556190	556188	556161
Templates	Cdk1	p130	Cyclin A	Cyclin E	bcl-x	p130
	Cdk2	Rb	Cyclin B	Cyclin F	p53	Rb
	Cdk3	p107	Cyclin C	Cyclin G1	GADD45	p107
	Cdk4	p53	Cyclin D1	Cyclin G2	c-fos	DP1
	p27	p57	Cyclin D2	Cyclin I	p21	DP2
	p21	p27	Cyclin D3	Cyclin H	bax	E2F1
	PISSLRE	p21	Cyclin A1	L32	bcl-2	E2F2
	p16	p19	L32	GAPDH	mcl-1	E2F4
	L32	p18	GAPDH		L32	L32
	GAPDH	p16			GAPDH	GAPDH
		p14/15				
		L32				
		GAPDH				

Mouse Cell Cycle Regulators

Mouse Complement

Template Set	mCC-1	mCYC-1	mCYC-2	mComplement
Catalog Number	559540	556241	556242	551490
Templates	cdk1	Cyclin A2	Cyclin E	C3aR
	cdk2	Cyclin B1	Cyclin F	C5aR
	cdk4	Cyclin C	Cyclin G1	C1qRp
	cdk5	Cyclin D1	Cyclin G2	Factor B
	cdk7	Cyclin D2	Cyclin I	C1q-A chain
	cdk8	Cyclin D3	Cyclin H	C3
	KKIALRE	Cyclin A1	L32	Factor D
	PCTAIRE3	Cyclin B2	GAPDH	Crry
	PCTAIRE1	L32		L32
	PITALRE/CHED	GAPDH		GAPDH
	PITALRE			
	PITSLRE			
	L32			
	GAPDH			

Human and Mouse Proto-Oncogenes

Human Fibroblast Growth Factors

Template Set	hMyc	mMyc	mFos/Jun	hFGF-1
Catalog Number	551984	556193	556194	556243
Templates	mad3	Sin3	c-jun	FGF-1
	rox	c-myc	jun-B	FGF-2
	N-myc	N-myc	jun-D	FGF-10
	L-myc	L-myc	c-fos	FGF-11
	mad4	b-myc	fos B	FHF-2
	max	max	fra-1	FHF-4
	mad	mad	fra-2	L32
	mixi1	mxi	L32	GAPDH
	sin3	mad3	GAPDH	
	c-myc	mad4		
	L32	mnt		
	GAPDH	L32		
		GAPDH		

Human and Mouse Angiogenesis

Template Set	hAngio-1	hAngio-2	hAngio-3	mAngio-1
Catalog Number	556232	556851	559900	551418
Templates	flt1	Endothelin RA	Edg1	flt1
	flt4	Endothelin RB	Edg2	flt4
	TIE	Endothelin RB-Like	Edg3	TIE
	Thrombin Rec.	Thrombin Rec.	GPR41	TIE2
	TIE2	PAR2	KIAA0001	Thrombin Rec.
	CD31	PAR3	FEG1	CD31
	Endoglin	PAFR	L32	VEGF-C
	Angiopoietin	H963-PAFR	GAPDH	Endoglin
	VEGF	PAFR-like		VEGF
	VEGF-C	L32		Angiopoietin-1
	L32	GAPDH		L32
	GAPDH			GAPDH

Composition of Available Template Sets *(continued)*

Human Origin Recognition Complexes

Rat Neurotrophins

Template Set	hOrc	rNT-1
Catalog Number	556248	556148
Templates	MCM2	βNGF
	MCM3	BDNF
	MCM4	GDNF
	MCM5	CNTF
	MCM6	NT3
	MCM7	NT4
	CDC18	L32
	ORC1	GADPH
	ORC2	
	ORC4	
	ORC5	
	ORC6	
	L32	
	GADPH	

Human and Mouse Developmental Genes

Template Set	hFrizzled	mBMP-1	mEFN-1	mEPHR-1	mGDF-1	mPax	mWnt-1	mWnt-2	mBMPR
Catalog Number	556247	559625	559009	559144	558832	556246	550925	550249	551786
Templates	fritz	BMP8A	ephrin B3	EPHA1	GDF-9	Pax-1	Wnt1	Wnt7a	ALK1
	SARP1	BMP7	ephrin B2	EPHA2	GDF-8	Pax-2	Wnt2	Wnt7b	KIR1/ALK2
	SARP2	BMP6	ephrin B1	EPHA3	GDF-6	Pax-3	Wnt3	Wnt8d	KIR5/ALK3
	smoothened	BMP5	ephrin A6	EPHA4	GDF-5	Pax-4	Wnt3a	Wnt10a	KIR2/ALK4
	frz1/FZD2	BMP4	ephrin A5	EPHA5	GDF-3	Pax-5	Wnt4	Wnt10b	KIR4/ALK5
	frizzled3	BMP3B	ephrin A4	EPHA6	GDF-1	Pax-6	Wnt5b	Wnt11	KIR6/ALK6
	FZD3	BMP3	ephrin A3	EPHA7	L32	Pax-7	Wnt6	Wnt13	ALK7
	frizzled5	BMP2	ephrin A1	EPHA8	GAPDH	Pax-8	L32	Wnt15	AVR2
	frizzled6	BMP1	L32	EPHB2		Pax-9	GAPDH	L32	AVR2B
	L32	BMP8B	GAPDH	EPHB3		L32		GAPDH	MIS2R
	GAPDH	L32		EPHB4		GAPDH			L32
		GAPDH		EPHB6					GAPDH
				L32					
				GAPDH					

Human DNA Repair Pathways

Template Set	hBER-1a	hBER-2	hDisR-1	hDSBR-1	hDSBR-2	hMMR	hNER-1	hNER-2	hNER-3
Catalog Number	550575	557279	550251	559011	559924	559624	557168	559010	559574
Templates	SMUG1	POLε	APC	LIM15	ATM	PMS1	XPG	hCSB	POLε
	OGG1	PARP	RB1	RAD50	NBS1	PMS2	DDB1	hXPB	RFCp140
	TDG	POLδ	BLM	RAD54	XRCC4	MSH2	XPC	hTFIIHp52	POLδ
	APEX	LIG3	WRN	RAD52	LIG4	MSH3	XPF	hTFIIHp44	LIG1
	UNG	LIG1	FANCC	MRE11	XRCC2	MSH6	RPAp70	hCSA	RFCp40
	MPG	XRCC1	FANCA	XRCC2	XRCC3	MSH5	DDB2	hcdk7	RFCp38
	NTHL1	POLβ	BRCA1	XRCC3	XRCC9	MYH	HHR23B	hCyclinH	RFCp37
	RPA4	FEN1	BRCA2	RAD51	Ku86 (XRCC5)	MLH1	XPA	hTFIIHp34	RFCp36
	MGMT	PCNA	WT1	RAD51B	DNA-PK (XRCC7)	L32	RPAp32	ERcc1	PCNA
	MBD4	L32	L32	RAD51C	Ku70 (XRCC6)	GAPDH	RPAp14	XPD	L32
	L32	GAPDH	GAPDH	RAD51D	L32		L32	L32	GAPDH
	GAPDH			L32	GAPDH		GAPDH	GAPDH	
				GAPDH					

Mouse and Rat DNA Repair Pathways

Human G Protein Coupled Receptors

Template Set	mBER-1	mBER-2	rBER-1	hGPR-1	hOrpR-1
Catalog Number	550250	550584	550732	557061	557060
Templates	OGG1	PARP	OGG1	GPR10	mrg
	TDG	POLδ	PARP	GPR9-6	OrphanR
	APEX	LIG3	APE	GPR8	PHS1-2
	UNG	LIG1	POLδ	GPR7	RDC1
	MPG	XRCC1	MPG	GPR6	WATM1
	NTHL1	FEN1	POLβ	GPR5	mas
	MGMT	POLβ	MGMT	GPR4	PMI
	L32	PCNA	PCNA	GPR1	c-1zf10
	GAPDH	L32	L32	GPR3	L32
		GAPDH	GAPDH	GPR2	GAPDH
				L32	
				GAPDH	

Human Signal Transduction and Transcriptional Activators

Template Set	hMAPK	hMKK	hMKKK	hSmad	hSOCS	hSTAT
Catalog Number	559471	559953	559870	559646	559927	558834
Templates	ERK1	MEK1	MAPKAPK2	Smad1	CIS	Stat1
	ERK2	MEK2	MAPKAPK3	Smad2	SOCS7	Stat2
	ERK3	MKK3	MAPKAPK5	Smad3	SOCS6	Stat3
	ERK3 rel	MKK4 (JNKK1)	MEKK3	Smad4	SOCS5	Stat4
	ERK5/ERK4	MEK5	MTK1/MEKK4	Smad5	SOCS3	Stat5A
	ERK6	MKK6 (SAPKK3)	MTK5/ASK	Smad6	SOCS2	Stat5B
	p38	MKK7 (JNKK2)	L32	Smad7	SOCS1	Stat6
	p38β	L32	GAPDH	Smad8	L32	L32
	p38δ	GAPDH		L32	GAPDH	GAPDH
	L32			GAPDH		
	GAPDH					

Human and Mouse Matrix Metalloproteinases

Template Set	hMMP-1	hMMP-2	mMMP-1	mMMP-2	hITG-1	hITG-2	mITG-3
Catalog Number	551274	551275	551276	551277	559928	550355	550356
Templates	MMP8	MMP8	MMP1	MMP1	ITGA1	ITGAe	ITGA2b
	MMP12	MMP12	MMP2	MMP12	ITGA2	ITGB4	ITGAd
	TIMP3	TIMP3	MMP3	MMP3	ITGA3	ITGA4	ITGAL
	MMP1	MMP1	MMP9	MMP9	ITGA4	ITGB5	ITGAm
	MMP2	MMP2	MMP8	MMP8	ITGA5	ITGA6	ITGAx
	MMP7	MMP3	TIMP4	MMP7	ITGA6	ITGB6	ITGB2
	MMP9	MMP14	TIMP3	TIMP3	ITGA7	ITGB7	ITGB3
	MMP13	TIMP1	TIMP1	TIMP1	ITGA8	ITGB8	ITGB7
	TIMP2	TIMP2	TIMP2	TIMP2	ITGA9	ITGAv	ITGB8
	L32	L32	L32	L32	ITGAv	L32	ITGAv
	GAPDH	GAPDH	GAPDH	GAPDH	ITGB1	GAPDH	L32
					L32		GAPDH
					GAPDH		

Human Integrins

Human Toxicology

Template Set	hTox-1b	hTox-2	hTox-3	hTox-4	hTLR-1
Catalog Number	550793	550794	552134	552135	551983
Templates	CYP2B6	FMO1	UGT1A1	GSTM3	TLR1
	NCP1	FMO2	UGT1A7	GSTM2	TLR2
	CYP2C8	FMO3	UGT1A10	GSTM1	TLR4
	CYP1A2	FMO4	UGT1A9	GSTM4	TLR5
	CYP2A6	FMO5	UGT1A6	GSTM5	TLR6
	CYP2C9	SULT2A1	UGT1A8	GSTA4	L32
	CYP2C19	SULT2B1	UGT1A4	GSTA2	GAPDH
	CYP3A4	SULT1C1	UGT2B4	GSTA3	
	CYP2D6	SULT1A	UGT2B15	L32	
	L32	L32	UGT2B7	GAPDH	
	GAPDH	GAPDH	L32		
			GAPDH		

Human Toll Like Receptors

Human and Mouse T-cell Receptor

Template Set	hTCR Vβ-A	hTCR Vβ-B	hTCR Vβ-C	mTCR Vβ-A	mTCR Vβ-B	mTCR Vβ-C
Catalog Number	552583	552586	552587	552588	552589	552590
Templates	Vβ16 (14s1)	Vβ14 (27s1)	Vβ20 (30s1)	Vβ2	Vβ 5	Vβ18
	Vβ18 (18s1)	Vβ21 (11s1)	Vβ3 (28s1)	Vβ11	Vβ14	Vβ5
	Vβ22 (2s1)	Vβ2 (20s1)	Vβ4 (29s1)	Vβ16	Vβ3	Vβ13
	Vβ5 (5s1)	Vβ13 (6s1)	Vβ11 (25s1)	Vβ12	Vβ20	Vβ8
	Vβ23 (13s1)	Vβ17 (19s1)	Vβ 2 (10s1)	Vβ1	Vβ6	Vβ10
	Vβ6 (7s3)	Vβ8 (12s3)	Vβ15 (24s1)	Vβ4	Vβ9	C2
	Vβ9 (3s1)	Vβ7 (4s1)	Vβ24 (15s1)	C2	Vβ7	C1
	C2	C2	Vβ1 (9s1)	C1	C2	
			C2		C1	

BD RiboQuant™ Custom Services

Custom Assembly of Template Sets

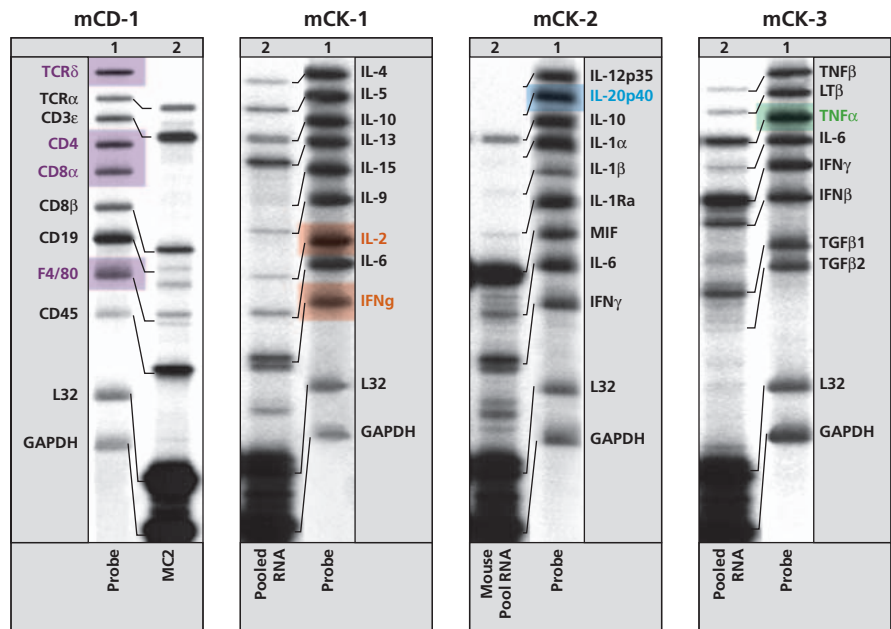
To ensure that your research needs are met, BD Biosciences Pharmingen makes the entire inventory of BD RiboQuant™ templates available for assembly into custom sets tailored to your individual experimental design. Custom Multi-Probe Template Sets can be assembled from any of the available templates, taking into consideration that each of the probes greater than 250 bp must differ by at least 25 nucleotides, and probes smaller than 250 bp by 12 nucleotides. The capacity of the system is 12 targets plus 2 housekeeping control genes. Please refer to the gene tables on pages 14-22 for the templates and probe sizes currently available.

An example of a research-specific custom set assembly.¹

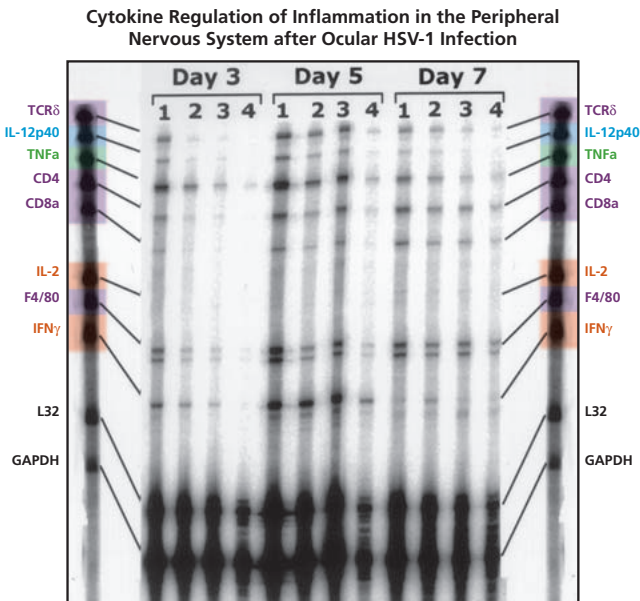
Ocular infection of Herpes Simplex Virus type-1 (HSV-1) in BALB/c mice results in transmission of the virus to the trigeminal ganglion, part of the peripheral nervous system. In the trigeminal ganglion the presence of viral antigens causes an inflammatory reaction which is mediated by leukocytes and cytokines. IFN γ and TNF α individually and in combination regulate the inflammatory reaction as evidenced by antibody neutralization experiments. The goal of this experiment was to monitor both cytokine gene expression and the effect of cytokines on leukocyte infiltration at the inflammatory site. Since the cytokine genes and the leukocyte-specific markers are not included in an existing Multi-Probe Template Set, a custom template set was designed for the detection of these specific genes. The Multi-Probe Template Set used in this study includes templates for mouse TCR δ , IL-12p40, TNF α , CD4, CD8 α , IL-2, F4/80 and IFN γ .

1. Kodukula P., Liu T., Van Rooijen N., Jager M., and Hendricks R. L. 1999. Macrophage control of Herpes Simplex Virus type 1 replication in the peripheral nervous system. *J. Immunol.* 162: 2895-2905

Existing Sets



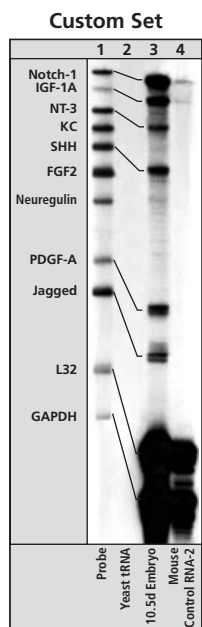
Custom Set using existing templates (see above)



Mice receiving different treatments: Control mAb (lane 1), anti-TNF α (lane 2), anti-IFN γ (lane 3) or anti-TNF α and anti-IFN γ (lane 4) were sacrificed 3, 5 and 7 days following HSV-1 infection. The trigeminal ganglion was dissected and total RNA extracted. RNase protection assay was performed using the custom template set. Shown above is an example of RPA data obtained by phosphorimager analysis.

Custom Cloning of Templates

In addition to our custom template assembly service, BD Biosciences Pharmingen also provides custom cloning. If a template is requested that is not in our inventory, we will design and generate the template based on available sequences. Using probe algorithms created at BD Biosciences, we select a region within the gene of interest that will provide optimal probe specificity. Using conventional cloning techniques, a template is generated, the sequence is confirmed, and the probe is tested by RPA. Custom templates may be assembled with appropriately-sized templates in a custom set.



Example of a custom template set where all genes were cloned and assembled according to the customer's request.

Custom RNase Protection Assay Services

The BD Biosciences Custom Products and Services Group (CPSG) provides RPA services for the detection and quantification of multiple mRNA species. Gene regulation in cultured cells and frozen tissue samples can be monitored for basic and clinical research studies.

Custom Services include:

RNA preparation and quantification.

RNA is isolated from samples using acid guanidinium thiocyanate-phenol-chloroform extraction. Total RNA is quantitated for use in RPA.

Probe synthesis, hybridization, and RNase treatment.

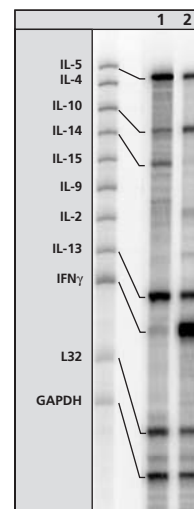
³²P-labeled, antisense RNA probes are generated from standard and customized BD RiboQuant Multi-Probe Template Sets and hybridized with target RNA. Free probe and other single-stranded RNA are digested with RNases.

Electrophoresis separation and autoradiogram.

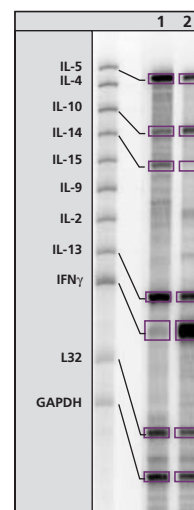
RNase protected probes are purified and resolved on denaturing polyacrylamide gels and developed on film. The expressed mRNA species are identified by the presence of bands corresponding to the expected fragment length.

Phosphorimaging quantification.

Radioactivity of ³²P-labeled probes are measured by phosphorimaging. The quantity of each mRNA species is determined based on the housekeeping genes L32 and GAPDH.



³²P-labeled anti-sense RNA probes hybridized to total RNA from samples 1 and 2 in RNase protection assay. Protected probes purified and resolved on denaturing polyacrylamide gel.



Selection of protected probes for quantitation of mRNA species detected by phosphorimaging. Quantitative comparison of cytokine mRNA levels in samples 1 and 2 based on intensity of ³²P-labeled probe fragments.

BD Ribonuclease Protection Assay Templates

Template	Probe	Protected	Template	Probe	Protected	Template	Probe	Protected
h ABC1	286	257	h CCL1 / I-309	190	161	h c-IAP-1 / MIHB	207	179
h alkaline phosphatase	173	144	h CCL7 / MCP-3	210	181	h c-IAP-2 / MIHC	231	203
h androgen receptor	429	400	h CCL8 / MCP-2	282	253	h CIS	315	287
h angiotensin II	192	163	h CCL13 / MCP-4	351	322	h CLARP/CASPER	282	253
h APC	429	400	h CCL14 / HCC-1	174	145	h collagen α 1 type III	231	204
h APE	324	295	h CCL15 / MIP-1 δ	174	145	h collagen α 1 type IV	210	181
h ara24	315	286	h CCL16 / HCC-4	189	163	h collagen α 2 type I	256	228
h ara54	284	255	h CCL17 / TACR	210	181	h cox1	316	287
h ara55	256	227	h CCL18 / PARC	231	202	h cox2	285	256
h ara70	231	202	h CCL19 / MIP-3 β	255	226	h CRADD	228	199
h ara160	394	320	h CCL20 / MIP-3 α	282	253	h CRAF (TRAF3)	177	148
h ASK1	390	361	h CCL21 / SLC	315	286	h CSA	255	226
h ATM	432	404	h CCL22 / MDC	351	322	h CSB	390	361
h bad	282	253	h CCL23 / MPIF-1	393	364	h CXCR1	390	364
h BAG-1	432	403	h CCL25 / TECK	435	406	h CXCR2	350	324
h bak	231	202	h CCR1	389	360	h CXCR3	315	287
h bax	210	181	h CCR2a	191	166	h CXCR4	285	257
h β c	283	254	h CCR2a+b	208	183	h cyclin A	315	286
h bcl-2	189	160	h CCR2b	173	144	h cyclin A1	174	145
h bcl-w	429	401	h CCR3	349	320	h cyclin B	282	253
h bcl-x (L)	393	364	h CCR4	314	287	h cyclin C	255	226
h bcl-x (S)	393	324	h CCR5 (A)	284	257	h cyclin D1	231	202
h bfl1	315	286	h CCR5 (B)	315	288	h cyclin D2	210	181
h bid	285	257	h CCR5 (B) del	315	255	h cyclin D3	189	160
h bik	255	226	h CCR5 (C)	315	287	h cyclin E	315	286
h BLM	349	320	h CCR6	429	400	h cyclin F	282	253
h BLR-1	232	204	h CCR7 / BLR-2	208	179	h cyclin G1	255	226
h BLR-2 / CCR7	208	179	h CCR8	255	227	h cyclin G2	231	203
h BRCA1	211	182	h CD1a	210	181	h cyclin H (A)	189	160
h BRCA2	189	160	h CD3e	315	287	h cyclin H (B)	210	181
h Btf2	350	321	h CD4	284	255	h cyclin I	210	182
h c-1zf10	210	181	h CD8 α	256	227	h CYP1A2	255	227
h c2	120	90	h CD8 β	231	202	h CYP2A6	231	202
h e-cadherin	389	360	h CD11b	284	255	h CYP2B6	390	361
h CART (TRAF4)	315	286	h CD14	190	162	h CYP2C8	285	256
h CAS	174	145	h CD19	209	184	h CYP2C9	190	161
h caspase-1	192	163	h CD31	231	202	h CYP2C19	171	142
h caspase-2 (S)	231	150	h CD34	208	179	h CYP2D6	153	125
h caspase-2 (L)	231	203	h CD45	165	137	h CYP3A4	171	142
h caspase-3	349	320	h CD56	349	320	h DAD1	345	316
h caspase-4	387	359	h CDC18	252	223	h DAP	174	145
h caspase-5	256	227	h cdk1	390	362	h DAP kinase	192	163
h caspase-6	312	283	h cdk2	347	321	h DAXX	192	163
h caspase-7	210	181	h cdk3	314	287	h DcR-1	315	286
h caspase-8	435	406	h cdk4	285	258	h DcR-2 (A)	199	170
h caspase-9	156	127	h cdk5 (A)	226	197	h DcR-2 (B)	383	357
h caspase-10	282	253	h cdk7 (C)	231	202	h DcR-3	345	317

h: human m: mouse r: rat b: bovine

Template	Probe	Protected
h DDB1	351	322
h DDB2	231	202
h DDX	390	361
h DFF	285	256
h DNA-PK	171	144
h DP1	315	286
h DP2	286	257
h Dr-nm23	231	202
h DR3	282	254
h DR4	231	202
h DR5	255	227
h DRM	161	133
h E2F1	255	226
h E2F2	231	203
h E2F4	192	164
h EBI3	173	144
h Edg1	312	283
h Edg2	282	253
h Edg3	255	226
h EGFR	285	258
h endoglin	210	181
h endothelin 1	285	256
h endothelin R A	429	400
h endothelin R B	393	364
h endothelin R B-like	354	325
h ENTG	231	202
h eotaxin	432	403
h ERCC1	171	142
h ERK1	459	430
h ERK2	417	388
h ERK3	378	349
h ERK3 rel	342	313
h ERK5 / ERK4	309	280
h ERK6 / p38 γ	279	250
h ETR101	172	143
h FADD	312	283
h FAF	231	202
h FANCA	231	202
h FANCC	255	227
h FAP	257	226
h FAS antigen	345	316
h FAS L	381	353
h FAST kinase	312	283
h FEG1	189	160
h FEN1	189	161
h FGF1	285	256
h FGF2	255	226

Template	Probe	Protected
h FGF3	231	202
h FGF4	210	181
h FGF5	189	161
h FGF6	171	144
h FGF7	315	286
h FGF8	285	256
h FGF9	255	226
h FGF10	231	203
h FGF11	210	181
h FGFR	222	193
h FHF2	189	160
h FHF4	171	142
h FLK1	369	340
h flt1	390	361
h flt4	348	319
h FMO1	432	404
h FMO2	390	361
h FMO3	351	322
h FMO4	324	295
h FMO5	285	256
h c-fos	255	226
h fritz	435	406
h frizzled 3	255	226
h frizzled 5	210	181
h frizzled 6	192	163
h frz1 (FZD2)	282	253
h F-SRC-1	190	161
h G-CSF	230	201
h G-CSFR	191	164
h GADD45	285	256
h GADD153	260	231
h GAPDH	124	96
h γ c	230	202
h α -globin	173	148
h β -globin	359	330
h γ -globin	309	280
h GLUT1	380	352
h GLUT2	345	317
h GLUT3	312	283
h GLUT4	282	253
h GLUT5	255	226
h GM-CSF	283	255
h GM-CSFR α	314	286
h GNB1	284	255
h gp130	231	202
h GPR1	209	180
h GPR2	177	148

Template	Probe	Protected
h GPR3	189	160
h GPR4	231	202
h GPR5	255	226
h GPR6	282	253
h GPR7	312	283
h GPR8	345	316
h GPR9-6	381	352
h GPR10	435	406
h GPR15	231	202
h GPR41	230	201
h Granulysin (A)	174	145
h Granulysin (B)	192	163
h Granzyme 3	210	181
h Granzyme A	432	403
h Granzyme B	390	361
h Granzyme H	282	253
h GSTA2	190	160
h GSTA3	172	142
h GSTA4	211	181
h GSTM1	287	257
h GSTM2	327	279
h GSTM3	350	321
h GSTM4	257	227
h GSTM5	232	202
h H963-PAFR	210	181
h Harakiri	345	316
h HCC-1 / CCL14	174	145
h HCC-4 / CCL16	189	163
h HGF	282	253
h HHR23B	210	183
h HIF-1 α	258	227
h hyaluronan synthase I	285	257
h hyaluronan synthase II	256	230
h hyaluronan synthase III	236	208
h I-309 / CCL1	190	161
h I-TRAF β	282	254
h ICAM-1	284	255
h IFN α RI	390	361
h IFN α RII	349	320
h IFN β	256	228
h IFN γ (A)	173	145
h IFN γ (B)	284	258
h IFN γ R α	209	180
h IFN γ R β	189	160
h IGF-I	350	321
h IGF-I R	435	408
h IGFBP-1	285	257

h: human m: mouse r: rat b: bovine

BD Ribonuclease Protection Assay Templates *(continued)*

Template	Probe	Protected	Template	Probe	Protected	Template	Probe	Protected
h IκBα	284	255	h ITGA5	309	280	h MAPKAPK2	393	364
h IL-1α	283	255	h ITGA6	279	250	h MAPKAPK3	351	322
h IL-1β	257	230	h ITGA7	252	223	h MAPKAPK5	315	286
h IL-1Rα	230	203	h ITGA8	228	199	h mas	255	226
h IL-1RI	389	360	h ITGA9	252	223	h max	255	226
h IL-1RII	349	320	h ITGAd	417	388	h mcl1	168	139
h IL-2	218	191	h ITGAe	417	388	h MCM2	459	430
h IL-2Rα	190	165	h ITGAL	378	349	h MCM3	417	388
h IL-2Rβ	209	180	h ITGAm	342	313	h MCM4	378	349
h IL-3	350	321	h ITGAv	189	160	h MCM5	342	313
h IL-3Rα	351	322	h ITGAX	309	280	h MCM6	309	280
h IL-4	351	325	h ITGB1	174	145	h MCM7	279	250
h IL-4Rα	257	228	h ITGB2	279	250	h MCP-1	232	203
h IL-5	388	360	h ITGB3	252	223	h MCP-2 / CCL8	282	253
h IL-5Rα	389	361	h ITGB4	378	349	h MCP-3 / CCL7	210	181
h IL-6 (B)	211	182	h ITGB5	309	280	h MCP-4 / CCL13	351	322
h IL-6 (C)	173	144	h ITGB6	252	223	h MDC / CCL22	351	322
h IL-6Rα	256	228	h ITGB7	228	199	h MDM 2	162	133
h IL-7	314	285	h ITGB8	207	178	h MDM X	177	148
h IL-7Rα	388	361	h JNK1	282	253	h MDR 1	345	316
h IL-8	210	181	h JNK2	210	181	h MEK1	393	364
h IL-9	231	203	h c-jun	231	202	h MEK2	351	322
h IL-9Rα	350	321	h Ki-67	349	320	h MEK5	255	226
h IL-10	316	287	h KIAA0001	209	180	h MEK6/MKK6	231	202
h IL-10Rα	389	361	h Ku70 (XRCC6)	158	127	h MEKK3	282	253
h IL-10Rα2	436	407	h Ku86 (XRCC5)	189	161	h MGMT	189	160
h IL-11R	350	323	h L32	141	113	h MIHB / c-IAP-1	207	179
h IL-12p35	389	360	h LDL receptor	233	206	h MIHC / c-IAP-2	231	203
h IL-12p40	349	320	h leukotriene A-4	256	227	h MIP-1α	256	228
h IL-12Rβ1	314	286	h leukotriene C4	233	204	h MIP-1β	315	286
h IL-12Rβ2	285	255	h LHCGR	316	287	h MIP-1δ / CCL15	174	145
h IL-13	190	161	h LIF	190	161	h MIP-3α / CCL20 (A)	284	255
h IL-13Rα	316	288	h LIFRα	210	182	h MIP-3α / CCL20 (B)	282	253
h IL-13Rα1	435	410	h LIG1	255	226	h MIP-3β / CCL19 (A)	172	143
h IL-14	283	256	h LIG3	285	258	h MIP-3β / CCL19 (B)	255	226
h IL-15	256	227	h LIG4	285	256	h mixi1	210	181
h IL-15Rα	285	256	h LIM15	432	404	h MKK3	315	286
h IL-18/IGIF	191	162	h 5-lipoxygenase	210	181	h MKK4 / JNKK1	282	253
h IL-18R	256	228	h 5-lipoxygenase act. prot.	192	165	h MKK7 / JNKK2	210	181
h IL-23 / p19	232	203	h LPA	210	181	h MLH-1	189	160
h IP-10	351	322	h LTβ	350	328	h MMP1	309	280
h IPL	435	407	h LTB-4R	315	286	h MMP2	280	250
h ITGA1	459	430	h Ltn	432	405	h MMP3	252	223
h ITGA2	417	388	h M-CSF	257	230	h MMP7	253	223
h ITGA2b	459	430	h mad3	430	401	h MMP8	460	430
h ITGA3	378	349	h mad4	285	258	h MMP9	228	199
h ITGA4	342	313	h MADD	180	151	h MMP12	379	349

h: human m: mouse r: rat b: bovine

Template	Probe	Protected	Template	Probe	Protected	Template	Probe	Protected
h MMP13	175	145	h p38 / Mxi2	252	223	h Rb	381	352
h MMP14	206	176	h p38β / SAP2β	228	199	h RB1	381	352
h MPG	255	226	h p38δ / SAPK4	207	178	h RDC1	315	286
h MPIF-1 / CCL23	393	364	h p53	312	283	h Requiem	192	163
h MRE11	285	258	h p57	281	252	h RFCp36	171	144
h mrg	435	406	h p107	345	317	h RFCp37	189	160
h MSH2	324	296	h p130	429	400	h RFCp38	209	183
h MSH3	285	256	h p450 aromatase	285	256	h RFCp40	231	203
h MSH5	231	202	h PAFR	231	202	h RFCp140	351	323
h MSH6	255	226	h PAFR-like	189	160	h RIP	162	134
h MTHF3	158	132	h PAI-1	350	321	h rox	381	352
h MTK1 / MEKK4	255	226	h PAR2	282	253	h RPA4	210	181
h MTK5 / ASK	231	202	h PAR3	255	226	h RPAp14	156	127
h MUC3	284	255	h PARC / CCL18	231	202	h RPAp32	170	143
h MUC5AC	189	160	h PARP	351	322	h RPAp70	255	227
h MUC5B	161	132	h PCNA (A)	171	142	h RVP1	255	227
h MUC6	210	181	h PCNA (B)	156	128	h SAP2β / p38β	228	199
h Mxi2 / CSAID / p38	252	223	h perforin	162	133	h SAPK4 / p38δ	207	178
h c-myc	162	133	h phospholipase A-2	349	320	h SARP1	390	361
h L-myc	312	284	h PHS1-2	354	325	h SARP2	351	322
h N-myc	345	316	h PISSLRE	191	162	h SCF	172	143
h MYH	210	181	h PKCα	312	283	h E-selectin	257	230
h NAIP	282	253	h plasminogen	191	162	h L-selectin	315	286
h NBS1	390	362	h PMI	231	202	h P-selectin	256	227
h NCPR	324	295	h PMS1	390	363	h SGLT1	231	202
h NFκB (p65)	386	357	h PMS2	351	323	h SGLT2	210	181
h NIP1	210	181	h POLβ	210	183	h SIAH	312	283
h NIP2	255	226	h POLδ	324	295	h sin3	192	163
h NIP3	231	202	h POLε	390	362	h SLC / CCL21	315	286
h ecNOS	285	256	h PRNP	315	286	h SMAD1	435	406
h iNOS	255	226	h Protein C	171	142	h SMAD2	390	361
h OGG1	390	361	h Protein S	192	163	h SMAD3	351	322
h ORC1	228	199	h RAC3	170	141	h SMAD4	312	283
h ORC2	207	178	h RAD50	390	362	h SMAD5	282	253
h ORC4	189	161	h RAD51	210	185	h SMAD6	255	226
h ORC5	174	145	h RAD51B	189	160	h SMAD7	231	202
h ORC6	162	133	h RAD51C	171	148	h SMAD8	209	181
h orphanR	393	364	h RAD51D	156	127	h smoothened	312	283
h OSM	155	128	h RAD52	324	297	h SMUG1	432	403
h osteocalcin	156	127	h RAD54	351	323	h SOCS1	174	145
h osteopontin	231	202	h RAGE	349	322	h SOCS2	191	165
h p14 / p15	159	133	h RANTES	388	361	h SOCS3	210	181
h p16	175	146	h c-raf	189	160	h SOCS5	231	202
h p18	191	163	h Ha-ras (C)	429	400	h SOCS6	252	226
h p19	210	182	h Ha-ras (D)	345	316	h SOCS7	282	253
h p21	231	202	h Ha-ras (E)	238	209	h SR-B1 / CLA-1	259	232
h p27	256	227	h K-ras	177	148	h STAT1	438	409

h: human m: mouse r: rat b: bovine

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BD Ribonuclease Protection Assay Templates *(continued)*

Template	Probe	Protected	Template	Probe	Protected	Template	Probe	Protected
h STAT2	390	361	h TNF α (A)	314	286	h V β 17 (19s1)	189	159
h STAT3	351	322	h TNF α (C)	436	407	h V β 18 (18s1)	257	227
h STAT4	312	283	h TNF β	388	359	h V β 20 (30s1)	333	303
h STAT5a	282	253	h TNFRp55 (A)	284	255	h V β 21 (11s1)	258	228
h STAT5b	255	226	h TNFRp55 (B)	189	160	h V β 22 (2s1)	231	201
h STAT6	231	202	h TNFRp75	315	287	h V β 23 (13s1)	189	159
h STRL33	435	408	h tPA	389	363	h V β 24 (15s1)	176	146
h SULT1A	174	145	h TRADD	177	149	h VBP1	209	181
h SULT1C1	210	181	h TRAF1 / EBI6	390	361	h VCAM-1	316	288
h SULT2A1	255	226	h TRAF2 / TRAP3	351	322	h VEGF	177	148
h SULT2B1	231	202	h TRAF3 / CRAF	177	148	h VEGF-C	165	136
h survivin	390	361	h TRAF4 / CART	315	286	h vWF	315	286
h syndecan-1	349	320	h TRAF5	255	226	h WATM1	282	253
h syndecan-4	314	286	h TRAF6	192	163	h XIAP	435	406
h TAC2	159	129	h I-TRAF β	282	254	h XPA	189	160
h TACE	349	320	h TRAIL	210	181	h XPB	351	322
h TACR / CCL17	210	181	h TRIP	165	136	h XPC	324	295
h TCR α	350	321	h TRPM2	192	163	h XPD	156	127
h TCR δ	389	361	h UB2N	256	227	h XPF	285	256
h TCR ζ	430	401	h UDG1	285	256	h XPG	390	364
h TDG	351	322	h US28	389	360	h XRCC1	231	202
h TECK / CCL25	435	406	h UGT1A1	392	362	h XRCC2	255	227
h TERT	360	321	h UGT1A4	197	167	h XRCC3	231	202
h TFIIHp34	189	160	h UGT1A6	257	227	h XRCC4	324	295
h TFIIHp44	285	256	h UGT1A7	339	319	h XRCC5 / Ku86	189	161
h TFIIHp52	324	295	h UGT1A8	219	189	h XRCC6 / Ku70	158	127
h TGF α	211	182	h UGT1A9	285	255	h XRCC9	210	181
h TGF β 1	189	160	h UGT1A10	334	294	h WRN	285	256
h TGF β 2	209	180	h UGT2B4	180	150	h WT1	171	142
h TGF β 3	232	206	h UGT2B7	150	120	m 6CKine	430	401
h TGF β RI	210	181	h UGT2B15	169	139	m ABC1	286	257
h TGF β RII	191	162	h V28	189	161	m AIC2A	350	322
h TGS-6	209	180	h V β 1 (9s1)	154	124	m AIC2B	209	180
h Thrombin R (A)	285	256	h V β 2 (20s1)	235	205	m ALK1	459	430
h Thrombin R (B)	315	286	h V β 3 (28s1)	306	276	m KIR1 / ALK2	417	388
h Thrombomodulin	231	202	h V β 4 (29s1)	270	240	m KIR5 / ALK3	378	349
h TIE	312	283	h V β 5 (5s1)	215	185	m KIR2 / ALK4	342	313
h TIE2	255	231	h V β 6 (7s3)	171	141	m KIR4 / ALK5	309	280
h TIMP1	169	140	h V β 7 (4s1)	160	130	m KIR6 / ALK6	279	250
h TIMP2	155	126	h V β 8 (12s3)	174	144	m ALK7	252	223
h TIMP3	324	313	h V β 9 (3s1)	145	115	m angiopoetin-1	165	136
h Tissue Factor	210	181	h V β 11 (25s1)	235	205	m APAF1	284	255
h TLR1	351	322	h V β 12 (10s1)	221	191	m Apex	324	295
h TLR2	324	295	h V β 13 (6s1)	208	178	m AVR2	228	199
h TLR4	255	226	h V β 14 (27s1)	286	256	m AVR2B	207	178
h TLR5	231	202	h V β 15 (24s1)	188	158	m bad	168	139
h TLR6	210	181	h V β 16 (14s1)	290	260	m bak	231	202

h: human m: mouse r: rat b: bovine

Template	Probe	Protected
m bax	210	182
m bcl-2	189	160
m bcl-w	435	406
m bcl-x (L)	299	272
m bcl-x (S)	299	239
m bfl1	391	364
m bid	178	149
m BLR-1	230	201
m BMP1	177	148
m BMP2	189	160
m BMP3	210	181
m BMP3B	231	202
m BMP4	255	226
m BMP5	282	253
m BMP6	312	283
m BMP7	345	316
m BMP8A	381	353
m BMP8B	162	133
m BMP9	172	143
m BMP10	189	160
m BMP11	209	180
m BMP15	232	202
m C1	122	92
m C1q-A chain	256	227
m C1q Receptor / C1qRp	315	286
m C2	137	107
m C2-GnT	285	256
m C3	230	201
m C3aR	391	362
m C5aR	351	322
m cannabinoid receptor 1	208	179
m cannabinoid receptor 2	172	143
m caspase-1	192	163
m caspase-2 (L)	231	202
m caspase-2 (S)	231	137
m caspase-3	354	325
m caspase-6	312	283
m caspase-7	210	181
m caspase-8	435	406
m caspase-11	282	253
m caspase-12	255	226
m caspase-14	180	151
m catalase	230	201
m CBFA1	192	163
m CC10	210	183
m CCR1	389	362
m CCR1b	351	322

Template	Probe	Protected
m CCR2	231	202
m CCR3	314	285
m CCR4	284	257
m CCR5 (B)	255	227
m CCR6	157	130
m CCR7	208	182
m CCR8 (A)	209	181
m CCR8 (B)	190	161
m CCR9	429	400
m CD3e	315	286
m CD4	284	255
m CD8 α	256	228
m CD8 β	231	202
m CD11b	259	230
m CD16	284	255
m CD19	209	180
m CD24	315	286
m CD31(B)	231	204
m CD31	232	202
m CDw32 / Fcy RII	314	285
m CD45	172	143
m cdk1	459	430
m cdk2	417	388
m cdk4	378	349
m cdk5	342	313
m cdk7	309	280
m cdk8	279	250
m cholesterol a-7-hydroxylase	209	180
m CLARP / FLIP	379	350
m collagen α 1 type I	231	205
m collagen α 1 type II	256	228
m collagen α 1 type X	286	257
m CRADD	228	199
m Cry	189	160
m CX3CR1	350	321
m CXCR2	351	323
m CXCR3	172	143
m CXCR4	284	255
m cyclin A1	174	145
m cyclin A2	315	286
m cyclin B1	282	253
m cyclin B2	162	133
m cyclin C	255	226
m cyclin D1	231	202
m cyclin D2	210	181
m cyclin D3	189	160
m cyclin E	315	286

Template	Probe	Protected
m cyclin F	282	253
m cyclin G1	255	226
m cyclin G2	231	203
m cyclin H	189	160
m cyclin I	210	182
m cyclophilin	190	161
m cytochrome C	189	160
m D1x5	286	257
m Daxx	210	181
m E2F1	314	285
m endoglin	192	163
m eotaxin (c)	156	127
m EPHA1	459	430
m EPHA2	417	388
m EPHA3	378	349
m EPHA4	342	313
m EPHA5	309	280
m EPHA6	279	250
m EPHA7	252	223
m EPHA8	228	199
m EPHB2	207	178
m EPHB3	195	166
m EPHB4	174	145
m EPHB6	162	133
m ephrin A1	169	142
m ephrin A3	188	160
m ephrin A4	210	181
m ephrin A5	231	202
m ephrin A6	255	226
m ephrin B1	282	253
m ephrin B2	312	283
m ephrin B3	345	316
m estrogen receptor α	211	182
m estrogen receptor β	158	129
m F4/80	190	163
m Factor B	282	253
m Factor D	210	181
m FADD	312	283
m FAF	231	202
m FAP	257	226
m FAS antigen	345	316
m FAS2L / TRAIL	210	181
m FASL	381	352
m fatty acid synthase	172	143
m FGF1	189	160
m FGF2	255	226
m flt1	390	361

h: human m: mouse r: rat b: bovine

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BD Ribonuclease Protection Assay Templates *(continued)*

Template	Probe	Protected	Template	Probe	Protected	Template	Probe	Protected
m flt4	348	319	m IL-2R γ	230	201	m c-Kit	172	144
m c-fos	312	287	m IL-3 (A)	191	163	m L32	141	112
m fosB	255	226	m IL-3 (B)	389	360	m LDL receptor	233	204
m fra1	231	202	m IL-3R α	284	256	m LIF	209	180
m fra2	210	181	m IL-4	390	364	m LIFR α	189	160
m Fuc-TIV/ELFT	349	320	m IL-4R α	255	226	m LT β	351	322
m Fuc-TVII	314	287	m IL-5	349	320	m LTB-4R	285	257
m γ C/IL-2R γ	230	201	m IL-5R α (A)	390	365	m Ltn (A)	390	361
m G-CSF	230	202	m IL-5R α (B) (membrane)	430	401	m Ltn (B)	480	451
m G-CSFR (B)	172	144	m IL-5R α (B) (soluble)	430	370	m M-CSF	257	231
m GAPDH	125	97	m IL-6 (A)	191	163	m mad	231	202
m GDF1	177	148	m IL-6 (B)	283	255	m mad3	192	163
m GDF3	189	160	m IL-6R α	256	227	m mad4	174	145
m GDF5	210	181	m IL-7 (D)	316	287	m MadCAM	316	287
m GDF6	255	226	m IL-7R α	390	361	m matrix gla protein	172	143
m GDF8	312	283	m IL-9	231	204	m max	255	226
m GDF9	345	316	m IL-9R α	349	320	m MCP-1 (A)	190	163
m α -globin	142	114	m IL-10	314	287	m MCP-1 (B)	210	181
m GM-CSF	283	257	m IL-10R	389	360	m MCP-3	258	229
m GM-CSFR α	315	287	m IL-11	351	322	m mdm2	389	362
m gp130 (C)	231	202	m IL-11R	351	323	m MDR	233	204
m HB-EGF	284	256	m IL-12p35 (A)	388	359	m Mgmt	210	181
m HGF	389	361	m IL-12p35 (B)	432	403	m MIF (A)	209	180
m HMG-1	312	283	m IL-12p40 (A)	349	321	m MIF (B)	157	128
m IBSP	314	285	m IL-12p40 (B)	156	127	m MIP-1 α	256	228
m ICAM-1 (A)	258	229	m IL-12R β	314	285	m MIP-1 β	285	257
m ICAM-1 (B)	435	406	m IL-12R β 2	269	241	m MIP-2	231	205
m IFN α -4	209	180	m IL-13	285	256	m MIP-3 α	157	128
m IFN β	232	203	m IL-13R α	315	286	m MIP-3 β	429	400
m IFN γ (A)	257	228	m IL-15	256	227	m MISR2	190	161
m IFN γ (C)	172	143	m IL-15R α	285	256	m MMP1	459	430
m IFN γ R α	209	180	m IL-18/IGIF	210	181	m MMP2	417	388
m IFN γ R β	189	161	m IP-10 (A) polymorphic	210	181	m MMP3	378	349
m IgERI α	255	227	m IP-10 (C)	215	181	m MMP7	279	250
m IgERI β	233	205	m IRF-1	349	320	m MMP8	309	280
m IgERI γ	209	180	m jagged	173	144	m MMP9	342	313
m IGF-IA	387	358	m JNK1	382	353	m MMP12	417	388
m I κ B α	284	255	m c-jun	429	400	m MnSOD	284	255
m IL-1 α	284	257	m junB	390	361	m mnt	162	133
m IL-1 β (A)	255	229	m junD	351	322	m MPG	255	226
m IL-1 β (B)	390	362	m KC (B)	315	286	m Msx2	257	228
m IL-1Ra	231	202	m KIR1 / ALK2	417	388	m mxi	210	181
m IL-1RI	390	361	m KIR2 / ALK4	342	313	m B-myc	282	253
m IL-1RII	349	320	m KIR4 / ALK5	309	280	m c-myc	381	352
m IL-2	204	181	m KIR5 / ALK3	378	349	m L-myc	312	284
m IL-2R α	191	162	m KIR6 / ALK6	279	250	m N-myc	345	316
m IL-2R β	209	182	m KKILARE	252	223	m neuregulin	231	202

h: human m: mouse r: rat b: bovine

Template	Probe	Protected
m iNOS (B)	434	406
m NFκB	231	202
m Notch-1	435	406
m NT-3	348	320
m Nth1	231	202
m ODC	157	129
m Ogg1	390	361
m OPG	210	181
m κ opioid receptor	190	161
m ν opioid receptor	229	200
m OTS-8 / gp38	209	180
m osteocalcin	210	181
m osteopontin	351	322
m p21 (A)	349	321
m p21 (B)	190	161
m p27	172	143
m p27 (B)	260	231
m p53 (A)	345	316
m p53 (B)	211	182
m p107	349	320
m p130	314	285
m PAR-2	231	190
m PARP	351	322
m pax1	390	362
m pax2	348	319
m pax3	312	283
m pax4	255	226
m pax5	231	202
m pax6	210	181
m pax7	192	163
m pax8	180	151
m pax9	168	139
m PCNA (A)	284	255
m PCTAIRE-1	207	178
m PCTAIRE-3	228	199
m PDGF-A	189	160
m PITAIRE/CHED	189	160
m PITALRE/Cdk9	174	145
m PITSLRE	162	133
m PKCα	231	205
m RANTES	349	321
m H-ras	190	162
m K-ras	157	132
m N-ras	210	184
m Rb	285	256
m RIP	162	133
m SCF	173	144

Template	Probe	Protected
m E-selectin	351	321
m SHH (sonic hedgehog)	285	256
m sin3	429	400
m SOCS1	230	201
m SOCS3	348	319
m SOX9	172	143
m SR-B1	259	231
m ST2L (membrane)	389	360
m ST2L (soluble)	389	321
m surfactant protein A	316	288
m surfactant protein B	284	255
m surfactant protein C	256	228
m surfactant protein D	231	202
m survivin	149	120
m TACE	349	320
m TCA-3	171	143
m TCRα	350	322
m TCRδ	389	360
m Tdg	351	324
m tenascin	172	144
m TGFα	232	204
m TGFβ1	208	179
m TGFβ2	191	162
m TGFβ3	171	143
m TGFβRI	210	181
m TGFβRII	191	162
m thrombin receptor	260	231
m TIE1	312	283
m TIE2	285	256
m TIMP1	207	178
m TIMP2	187	158
m TIMP3	228	199
m TIMP4	251	222
m TNFα (A)	316	287
m TNFα (B)	435	407
m TNFβ	389	361
m TNFRp55 (A)	283	254
m TNFRp55 (B)	189	160
m TNFRp75	314	285
m TRADD	174	145
m TRANCE	233	205
m thrombospondin / TSP-1	435	406
m Ung	285	256
m Vβ1	180	150
m Vβ2	315	285
m Vβ3	235	205
m Vβ4	160	130

Template	Probe	Protected
m Vβ5	270	240
m Vβ6	200	170
m Vβ7	155	125
m Vβ8	195	165
m Vβ9	178	178
m Vβ10	165	135
m Vβ11	270	240
m Vβ12	205	175
m Vβ13	220	190
m Vβ14	310	280
m Vβ15	325	295
m Vβ16	225	195
m Vβ18	310	280
m Vβ20	220	190
m VCAM-1	285	256
m VEGF	177	148
m VEGF-C	210	181
m Wnt1	381	352
m Wnt2	345	316
m Wnt3	312	283
m Wnt3a	282	253
m Wnt4	255	226
m Wnt5b	210	181
m Wnt6	192	163
m Wnt7a	435	406
m Wnt7b	390	361
m Wnt8d	351	322
m Wnt10a	312	283
m Wnt10b	282	253
m Wnt11	255	226
m Wnt13	210	181
m Wnt15	195	166
r ANF	255	226
r APE	324	295
r aquaporin-5	189	160
r bax	210	181
r bcl-2	189	160
r bcl-x (L)	393	363
r bcl-x (S)	393	325
r BDNF	315	286
r BMP2	342	313
r BMP3	309	280
r BMP4	417	388
r BMP6	378	349
r BMP7	174	145
r BNP	282	253
r caspase-1	282	253

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BD Ribonuclease Protection Assay Templates *(continued)*

Template	Probe	Protected	Template	Probe	Protected	Template	Probe	Protected
r caspase-2 (L)	231	202	r LDH-A	350	322	r TNFβ	351	322
r caspase-2 (S)	231	137	r LDH-B	390	361	r t-PA (B)	315	286
r caspase-3	255	226	r LDL-receptor	256	227	r trk	464	435
r catalase	255	226	r liver catalase	348	319	r tyrosine hydroxylase	192	163
r caveolin	210	181	r LTβ	210	181	r VEGF	418	389
r CB-1 (A)	311	282	r M1 Muscarinic Acetylcholine R	314	285	p AMCF-II	316	287
r CB-1 (B)	190	162	r M3 Muscarinic Acetylcholine R	168	139	p endothelin	431	403
r CB-2 (A)	380	351	r M4 Muscarinic Acetylcholine R	285	256	p G-CSF	257	228
r CB-2 (B)	222	193	r M5 Muscarinic Acetylcholine R	259	230	p GAPDH	125	96
r CD147	316	287	r MCP-1 (A)	171	142	p GM-CSF	284	256
r CNTF	255	226	r MCP-1 (B)	390	361	p ICAM	350	321
r collagen α1 type I	279	250	r MCT1	209	180	p IFNβ	232	203
r collagen α1 type II	229	200	r MCT4/3	255	226	p IFNγ	172	143
r collagen α1 type X	162	133	r MGMT	210	181	p IL-1α	284	255
r COX1	285	256	r MIF	171	142	p IL-1β	257	231
r COX4	231	202	r MIP-2	232	203	p IL-2	208	179
r CTGF	344	315	r MnSOD	284	255	p IL-4	388	365
r DBH	389	360	r MPG	255	226	p IL-6	191	162
r FAS antigen	435	406	r Muscarinic cholinergic R	209	180	p IL-8	191	162
r FASL	315	286	r NGFβ	351	322	p IL-10	316	288
r fibronectin	378	349	r NGFRp75	432	403	p IL-12p35	387	361
r GAPDH	126	97	r iNOS	210	181	p IL-12p40	351	325
r GDNF	282	253	r NT3	231	202	p IL-15	257	228
r γ-glutamylcysteine synth	315	286	r NT4	213	184	p IL-18	208	180
r glutathione peroxidase	209	180	r OGG1	390	361	p L32	140	114
r glutathione reductase	286	257	r osteocalcin	189	160	p MCP-1	231	204
r GM-CSF	324	295	r osteopontin	208	179	p MCP-2	208	180
r HMG-CoA reductase	256	183	r PAI-1	218	189	p iNOS	469	440
r IBSP	252	223	r PARP	351	322	p RANTES	172	144
r IFNβ	390	361	r PCNA	169	140	p TGFβ2	208	180
r IFNγ	156	127	r PDGF-B	350	321	p TNFα	316	288
r IL-1α	432	403	r phos hydro glut peroxidase	231	202	p TNFβ	390	362
r IL-1β	390	361	r PNMT	172	143	p VEGF	351	322
r IL-1Ra	351	322	r POLβ	231	201	b GAPDH	125	96
r IL-2	171	142	r POLδ	285	256	b GM-CSF	191	162
r IL-3	315	286	r RTI40	231	202	b IFN γ	158	135
r IL-4	285	256	r SOD1	187	158	b IL-1β	256	227
r IL-5	255	226	r SOD2	256	227	b IL-2	172	143
r IL-6	231	202	r surfactant protein A	255	226	b IL-4	435	405
r IL-9	432	404	r surfactant protein B	282	253	b IL-5	390	361
r IL-10	210	181	r surfactant protein C	315	286	b IL-6	231	202
r IL-12p35	323	294	r TGFβ1	285	256	b IL-8	210	181
r IL-12p40	285	256	r TGFβ2	231	202	b IL-10	285	256
r IL-15	189	160	r TGFβ3	255	227	b IL-12p35	352	322
r IL-18 / IGIF	255	226	r TH	192	163	b L32	141	112
r KC	156	127	r TIMP-1	202	173	b TNFα	316	287
r L32	141	112	r TNFα	189	160			

h: human m: mouse r: rat b: bovine

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