

Technical Data Sheet

Polyclonal Rabbit Anti-Human Fractin

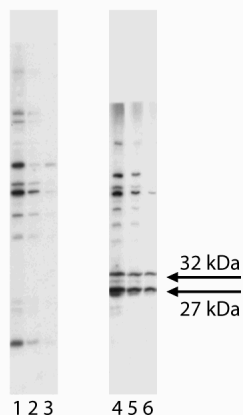
Product Information

Material Number:	551527
Alternate Name:	Cleaved Actin
Size:	100 µl
Clone:	Polyclonal
Immunogen:	Human β-actin 240-245
Isotype:	Rabbit Ig
Reactivity:	QC Testing: Human
Target MW:	27-32 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

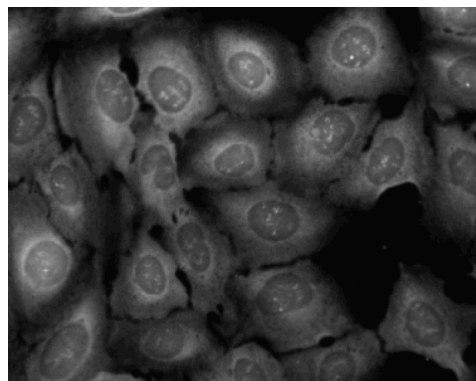
Description

Apoptosis is essential in maintaining cellular homeostasis and for normal development. Morphological as well as biochemical changes are a hallmark of apoptosis, and include membrane blebbing, chromosomal condensation and DNA fragmentation. These changes are caused by a class of cysteine proteases called caspases, which upon activation, can cleave multiple cytoplasmic and nuclear substrates. Actin is a 45 kDa caspase substrate that has been shown to be cleaved by caspase-3 to generate protein fragments ranging in size from ~32 to ~15 kDa in size. The cleavage of actin leads to cytoskeletal breakdown, release of DNase I and ultimately cleavage of DNA into fragments that are present during the late stages of apoptosis. Moreover, additional studies have shown that the cleavage of actin may play a role in the pathogenesis of Alzheimer's disease. The relationship between the in situ detection of cleaved actin in cell bodies of degenerating neurons and plaque-associated microglia in the pathophysiology of Alzheimer's disease remains to be elucidated.

It has been reported that the antibody recognizes the 32 kD cleaved fragment of human actin and does not recognize intact actin. A synthetic peptide (K-YELPD) representing β-actin residues 240-245 coupled by the K-residue to a protein derivative of tuberculin was used as the immunogen. The antibody has reportedly been useful for the immunohistochemistry application.



Western blot analysis of Fractin. SH-SY5Y cells (Human neuroblastoma; ATCC CRL-2266) were treated with 10 µM retinoic acid for 5 days to induce differentiation and then were either left untreated (lanes 1-3) or treated with 1 µM colchicine for 2 days to induce apoptosis. Cells were washed twice in PBS and lysed. Lysates were probed with the polyclonal rabbit anti-Fractin antibody at dilutions of 1:1000 (lanes 1,4), 1:2000 (lanes 2,5), and 1:4000 (lanes 3,6). Fractin is identified as bands of 32 and 27 kDa in treated cells (lanes 4-6) but absent in untreated cells (lanes 1-3). Similar results are also obtained with 4-6 µM camptothecin treated Jurkat cell lysates (Human T-cell leukemia; ATCC TIB-152).



Immunofluorescent staining of U2OS cells. Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10 000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure) and the anti-Fractin antibody. The second step reagent was FITC goat anti-rabbit Ig (Cat. No. 554020). Images were taken on a Pathway 850 imager using a 20x objective. This antibody also stained A549 and HeLa cells and worked with both the Triton X100 and Methanol fix/perm protocols (see Recommended Assay Procedure).

Preparation and Storage

Store undiluted at 4°C.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit bdbiosciences.com/how_to_order/

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2008 BD



Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Immunohistochemistry	Reported

Recommended Assay Procedure:

Western blot: Please refer to http://www.bdbiosciences.com/pharming/en/protocols/Western_Blotting.shtml

Bioimaging:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
554020	FITC Goat Anti-Rabbit Ig	0.5 mg	(none)
554021	HRP Goat Anti-Rabbit Ig	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
3. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.

References

Mashima T, Naito M, Noguchi K, Miller DK, Nicholson DW, Tsuruo T. Actin cleavage by CPP-32/apopain during the development of apoptosis. *Oncogene*. 1997; 14(9):1007-1012.(Biology)

Rossiter JP, Anderson LL, Yang F, Cole GM. Caspase-cleaved actin (fractin) immunolabelling of Hirano bodies. *Neuropathol Appl Neurobiol*. 2000; 26(4):342-346. (Biology: Immunohistochemistry)

Wolf BB, Green DR. Suicidal tendencies: apoptotic cell death by caspase family proteinases. *J Biol Chem*. 1999; 274(29):20049-20052.(Biology)

Yang F, Sun X, Beech W, et al. Antibody to caspase-cleaved actin detects apoptosis in differentiated neuroblastoma and plaque-associated neurons and microglia in Alzheimer's disease. *Am J Pathol*. 1998; 152(2):379-389.(Immunogen: Immunohistochemistry)