Technical Data Sheet

Purified Mouse Anti- Human PARP

Product Information

Material Number: 556494
Size: 0.1 mg
Concentration: 0.5 mg/ml
Clone: 4C10-5
Immunogen: Human PARP
Isotype: Mouse IgG1
Reactivity: QC Testing: Human
Target MW: 113 kD & 89 kD
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

PARP [Poly(ADP-ribose) polymerase] is a 113 kDa nuclear chromatin-associated enzyme that catalyzes the transfer of ADP-ribose units from NAD+ to a variety of nuclear proteins including topoisomerases, histones, and PARP itself. The catalytic activity of PARP is increased in non-apoptotic cells following DNA damage, and PARP is thought to play an important role in mediating the normal cellular response to DNA damage. Additionally, PARP is a target of the caspase protease activity associated with apoptosis. During apoptosis, PARP is cleaved from a 113 kDa intact form into smaller 89 kDa and 24 kDa fragments. This process separates the amino-terminal DNA-binding domain of the enzyme from the carboxy-terminal catalytic domain resulting in the loss of normal PARP function. Although the role of PARP in apoptosis remains to be elucidated, PARP cleavage is considered to be a marker of apoptosis. The 4C10-5 antibody recognizes both the intact 113 kDa form and 89 kDa fragment of PARP.

The 4C10-5 antibody has been reported to recognize both native and denatured PARP. Purified human PARP was used as the immunogen and the antibody reported to react with an epitope located in the NAD binding domain. In dot blot assays, the antibody reacts with the native enzyme in the presence or absence of bound DNA as well as after synthesis of covalently linked poly (ADP-ribose). The 4C10-5 antibody is routinely tested by Western blot analysis of untreated Jurkat T cells and Jurkat T cells induced to undergo apoptosis.

This antibody is routinely tested by Western blot analysis and immunofluorescent imaging. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

Left: Western blot analysis of PARP cleavage. Jurkat cells were untreated (lane 1) or induced to undergo Fas mediated apoptosis by treatment with anti-human Fas mAb, clone DX2 (cat. No. 556670) and Protein G for 4 hr (lane 2) and probed with the PARP antibody at 1-2 µg/ml (clone 4C10-5). The 113 kDa intact form of PARP is seen in both the untreated and Fas mAb-treated cell lysates. However, the 89 kDa PARP cleavage fragment is only seen in the treated cell lysates. Right: 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-PARP antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a Pathway 850 imager using a 20x objective. This antibody also stained A549 and HeLa cells using both the Triton X100 and methanol fix/perm protocols (see Recommended Assay Procedure).
Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. Store undiluted at 4° C.

Application Notes

<table>
<thead>
<tr>
<th>Application</th>
<th>Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioimaging</td>
<td>Routinely Tested</td>
</tr>
<tr>
<td>Western blot</td>
<td>Routinely Tested</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Reported</td>
</tr>
<tr>
<td>Immunoprecipitation</td>
<td>Reported</td>
</tr>
<tr>
<td>Dot Blot</td>
<td></td>
</tr>
</tbody>
</table>

Recommended Assay Procedure:

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

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>554002</td>
<td>HRP Goat Anti-Mouse Igs</td>
<td>1.0 ml</td>
<td>none</td>
</tr>
<tr>
<td>550959</td>
<td>Jurkat Apoptotic Lysate Set I</td>
<td>25 mg</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>554001</td>
<td>FITC Goat Anti-Mouse Igs</td>
<td>0.5 mg</td>
<td></td>
</tr>
</tbody>
</table>

Product Notices

1. 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.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

References