Technical Data Sheet

BD Horizon™

PE-CF594 Mouse anti-Cleaved PARP (Asp 214)

Product Information

Material Number: 564130
Alternate Name: Asp214
Size: 100 tests
Vol. per Test: 5 µl
Clone: F21-852
Immunogen: Human cleaved PARP
Isotype: Mouse IgG1, κ
Reactivity: QC Testing: Human
Tested in Development: Mouse

Storage Buffer:
Aqueous buffered solution containing BSA and ≤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 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. The PARP protein consists of an N-terminal DNA-binding domain (DBD) and a C-terminal catalytic domain separated by a central automodification domain. During apoptosis, Caspase-3 cleaves PARP at a recognition site (Asp Glu Val Asp Gly) in the DBD to form 24- and 89-kDa fragments. This process separates the DBD (which is mostly in the 24-kDa fragment) from the catalytic domain (in the 89-kDa fragment) of the enzyme, resulting in the loss of normal PARP function. It has been proposed that inactivation of PARP directs DNA-damaged cells to undergo apoptosis rather than necrotic degradation, and the presence of the 89-kDa PARP cleavage fraction is considered to be a marker of apoptosis.

A peptide corresponding to the N-terminus of the cleavage site (Asp 214) of human PARP was used as the immunogen. The F21-852 monoclonal antibody reacts only with the 89-kDa fragment of human PARP-1 that is downstream of the Caspase-3 cleavage site (Asp214) and contains the automodification and catalytic domains. It does not react with intact human PARP-1. Cross-reactivity with other members of the PARP superfamily is unknown. Recognition of cleaved PARP in mouse cells has been demonstrated, and it may also cross-react with a number of other species due to the conserved nature of the molecule.

This antibody is conjugated to BD Horizon™ PE-CF594, which has been developed exclusively by BD Biosciences as a better alternative to PE-Texas Red®. PE-CF594 excites and emits at similar wavelengths to PE-Texas Red® yet exhibits improved brightness and spectral characteristics. Due to PE having maximal absorption peaks at 496 nm and 564 nm, PE-CF594 can be excited by the blue (488-nm), green (532-nm) and yellow-green (561-nm) lasers and can be detected with the same filter set as PE-Texas Red® (eg 610/20-nm filter).

Flow cytometric analysis of Cleaved PARP (Asp 214) expression in Camptothecin-treated Jurkat cells. Cells from the human Jurkat (Acute T cell leukemia, ATCC TIB-152) cell line were cultured for 6 hours with (solid line histogram) or without (dashed line histogram) Camptothecin (Sigma-Aldrich Cat. No. C-9911; 12 µM final concentration). The cells were harvested, washed with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656), and fixed and permeabilized with BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (Cat. No. 554722). The cells were then washed and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with BD Horizon™ PE-CF594 Mouse Anti-Cleaved PARP (Asp 214) antibody (Cat. No. 564130). Histograms were derived from gated events with the forward and side light-scatter characteristics of intact Jurkat cells. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.
Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with BD Horizon™ PE-CF594 under optimum conditions, and unconjugated antibody and free PE-CF594 were removed.

Application Notes

Application

Intracellular staining (flow cytometry) Routinely Tested

Suggested Companion Products

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<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tbody>
<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 ml</td>
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<tr>
<td>554714</td>
<td>BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit</td>
<td>250 tests</td>
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<tr>
<td>554723</td>
<td>Perm/Wash Buffer</td>
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<td>(none)</td>
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<tr>
<td>562292</td>
<td>PE-CF594 Mouse IgG1, κ Isotype Control</td>
<td>0.1 mg</td>
<td>X40</td>
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Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
6. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
7. When excited by the yellow-green (561-nm) laser, the fluorescence may be brighter than when excited by the blue (488-nm) laser.
8. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using multi-laser cytometers, which may directly excite both PE and CF™594.

References