Technical Data Sheet

Purified Mouse Anti- FEN-1

Product Information

Material Number: 611294
Size: 50 µg
Concentration: 250 µg/ml
Clone: 21/FEN-1
Immunogen: Human FEN-1 aa. 252-371
Isotype: Mouse IgG1

Reactivity:
QC Testing: Human
Tested in Development: Dog, Mouse, Rat

Target MW: 50 kDa
Storage Buffer: Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

The fidelity of DNA replication, recombination, and repair is essential for genome stability. Proteins that have exonuclease and endonuclease enzymatic activity are critical for accurate replication, recombination, and repair of DNA sequences. FEN-1 (five’ exonuclease-1 or flap endonuclease-1) is an exo- and endonuclease found in yeast, mouse, and human. The 5'-3' exonuclease activity of FEN-1 involves only double stranded DNA and is important for processing Okazaki fragments during lagging strand DNA synthesis. In addition, FEN-1 is a member of the RAD2 family of repair nucleases and may participate in DNA repair. Its endonuclease activity is specific for 5’ overhanging flaps, which are intermediates in the repair of DNA double strand breaks. Human and mouse FEN-1 are highly conserved with 95% identity at the amino acid level. FEN-1 expression is highest during entry into the mitotic cell cycle and lowest in differentiated cells. Thus, FEN-1 is an exo- and endonuclease that is thought to be essential for maintaining DNA integrity during replication, as well as after damage by alkylating agents or UV.

This antibody is routinely tested by Western blot analysis and immunofluorescent staining. Other applications were tested at BD Biosciences Pharmingen during antibody development only.

Western blot analysis of FEN-1 on a human endothelial lysate. Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of the FEN-1 antibody.

Immunofluorescent staining of HeLa 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 Triton X100 fixperm protocol (see Recommended Assay Procedure) and the anti-FEN-1 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). Images were taken on a Pathway 850 imager using a 20x objective. This antibody also stained A549 and U2OS cells and worked with both the Triton X100 and Methanol fixperm 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 -20° C.

Application Notes

Application

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<tr>
<th>Application</th>
<th>Remarks</th>
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<tbody>
<tr>
<td>Bioimaging</td>
<td>Routinely Tested</td>
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<tr>
<td>Western blot</td>
<td>Routinely Tested</td>
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<tr>
<td>Immunofluorescence</td>
<td>Tested During Development</td>
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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>
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<tbody>
<tr>
<td>611450</td>
<td>Human Endothelial Cell Lysate</td>
<td>500 µg</td>
<td>(none)</td>
</tr>
<tr>
<td>554002</td>
<td>HRP Goat Anti-Mouse Igs</td>
<td>1.0 ml</td>
<td>(none)</td>
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<tr>
<td>554001</td>
<td>FITC Goat Anti-Mouse Igs</td>
<td>0.5 mg</td>
<td>Polyclonal</td>
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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.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Hosfield DJ, Mol CD, Shen B, Tainer JA. Structure of the DNA repair and replication endonuclease and exonuclease FEN-1: coupling DNA and PCNA binding to FEN-1 activity. Cell. 1998; 95(1):135-146.(Biology)