TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Anti-FEN-1 Polyclonal Antibody

Catalog #: 4410-PC-100 **Volume:** 100 µl

Description: Rabbit polyclonal antibody to mammalian flap-endonuclease (FEN-1). It is involved in DNA double-strand break repair and base excision repair. This antibody has been shown to inhibit FEN-1 activity.

Immunogen: Full-length recombinant human FEN-1 protein.

Physical state: This antibody is provided as rabbit anti-sera.

Specificity: The antibody detects human and mouse FEN-1.

Storage Conditions: Stable for at least 1 year at -20°C in a manual defrost freezer. Avoid repeated freezing and thawing by aliquoting into microtubes and storing at -20°C or -80°C.

Applications: For Western blotting, an antibody dilution of 1:1000 is suggested, but empirical testing will be required for optimal results.

Fig. 1. WEHI-1 mouse cells were treated with 25 µM etoposide and lysed in modified RIPA buffer (see reverse). Approximately 2 x 10^6 cells were analyzed by SDS-PAGE and Western blotting. Immunodetection was performed using a 1:1000 dilution of α-FEN-1 and chemiluminescence.

References:

1. Harrington, J.J. and M.R. Lieber. 1994. The characterization of a mammalian DNA structure-specific endonuclease. EMBO **13**:1235-1246.
2. Harrington, J.J. and M.R. Lieber. 1994. Functional domains within FEN-1 and RAD2 define a family of structure-specific endonucleases: implications for nucleotide excision repair. Genes and Development **8**:1344-1355.

 E9/22/10v1

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Cell Lysates for Western Blotting:
To prepare total cell lysates, solubilize cells in 1X modified RIPA buffer (50 mM Tris-HCl (pH 7.4), 1% NP-40, 0.25% Na-deoxycholate, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride) at 1 x 10^7 cells per ml. Add appropriate amount of SDS-PAGE loading buffer to cell extracts and heat for 5 minutes in a boiling water bath. Run samples on SDS polyacrylamide gel or store at -20°C.

Procedure for Immunoblotting using Peroxidase Detection:
Blotting buffer: 12 mM Tris base, 96 mM glycine, and 20% MeOH.
Blocking solution: 5% (w/v) nonfat dry milk in PBS.
Antibody solution: 5% (w/v) nonfat dry milk, 0.05% Tween in PBS.

Following electrophoresis, transfer the separated proteins to a PVDF or nitrocellulose membrane. After transfer, incubate the membrane for 30 minutes at room temperature in blocking solution.

Incubate the membrane in antibody solution containing 1:1000 dilution of anti-FEN-1 rabbit polyclonal antibody overnight at 4°C. Empirical determination of primary antibody concentration may be required for optimal results.

Wash the membrane 3 times in large amounts of 0.05% Tween-20 in PBS (antibody solution) at room temperature for 15 minutes. Changing the membrane containers along with the solution often reduces background.

Incubate the membrane at room temperature for 1 hour in a dilution of anti-rabbit antibody conjugated to horseradish peroxidase (HRP). Empirical determination of secondary antibody concentration will be required for optimal results.

Wash the membrane with 3 changes of 0.05% Tween-20 in PBS for 15 minutes.

Develop peroxidase reaction using chemiluminescence, or as described in the figure 2 legend.

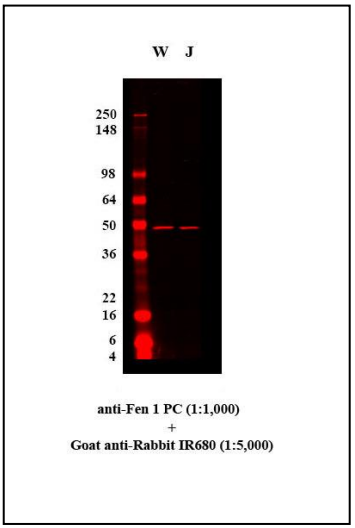

Fig. 2. Western blot analysis of Wehi and Jurkat cells using Trevigen's anti-FEN-1 antibody. Cells were lysed in Tris-Glycine SDS sample buffer at the concentration 1 x 10^7 cells/ml and 10 µl of each lysate were loaded per well of 4-20% Tris-Glycine gel. Proteins were transferred onto an Immobilon FL membrane and proteins were detected with Trevigen's anti-FEN-1 antibody (cat# 4410-PC-100) followed by IR680-conjugated secondary antibody (Licor). Membranes were scanned using an Odyssey Infrared Imaging System (Licor).

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