

Anti-FEN1 Antibody Picoband™ (monoclonal, 6F3F2)

Catalog # ABO16236

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P39748
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-FEN1 Antibody Picoband™ (monoclonal, 6F3F2) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	2237
Other Names	Flap endonuclease 1 {ECO:0000255 HAMAP-Rule:MF_03140}, FEN-1 {ECO:0000255 HAMAP-Rule:MF_03140}, 3.1.-.- {ECO:0000255 HAMAP-Rule:MF_03140}, DNase IV, Flap structure-specific endonuclease 1 {ECO:0000255 HAMAP-Rule:MF_03140}, Maturation factor 1, MF1, hFEN-1, FEN1 {ECO:0000255 HAMAP-Rule:MF_03140}, RAD2
Calculated MW	42593
Application Details	Western blot, 0.25-0.5 µg/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na ₂ HPO ₄ .
Clone Names	Clone: 6F3F2
Immunogen	E.coli-derived human FEN1 recombinant protein (Position: Q4-E300).
Purification	Immunogen affinity purified.
Storage	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.

Protein Information

Name	FEN1 {ECO:0000255 HAMAP-Rule:MF_03140}
Synonyms	RAD2
Function	Structure-specific nuclease with 5'-flap endonuclease and 5'-3' exonuclease activities involved in DNA replication and repair. During DNA replication, cleaves the 5'-overhanging flap structure that is generated by displacement synthesis when DNA polymerase encounters the 5'-end of a downstream Okazaki fragment. It enters the flap from the 5'-end and then tracks to cleave the flap base, leaving a nick for ligation. Also involved in the long patch base excision repair (LP-BER) pathway, by cleaving within the apurinic/aprimidinic (AP) site-terminated flap. Acts as a genome stabilization factor that prevents flaps from equilibrating into structures that lead to duplications and deletions. Also possesses 5'-3' exonuclease activity on nicked or gapped double-stranded DNA, and exhibits RNase H activity. Also involved in replication and repair of rDNA and in repairing mitochondrial DNA.
Cellular Location	[Isoform 1]: Nucleus, nucleolus. Nucleus, nucleoplasm. Note=Resides mostly in the nucleoli and relocalizes to the nucleoplasm upon DNA damage

Background

Flap endonuclease 1 is an enzyme that in humans is encoded by the FEN1 gene. It is mapped to 11q12.2. The protein encoded by this gene removes 5' overhanging flaps in DNA repair and processes the 5' ends of Okazaki fragments in lagging strand DNA synthesis. Direct physical interaction between this protein and AP endonuclease 1 during long-patch base excision repair provides coordinated loading of the proteins onto the substrate, thus passing the substrate from one enzyme to another. The protein is a member of the XPG/RAD2 endonuclease family and is one of ten proteins essential for cell-free DNA replication. DNA secondary structure can inhibit flap processing at certain trinucleotide repeats in a length-dependent manner by concealing the 5' end of the flap that is necessary for both binding and cleavage by the protein encoded by this gene. Therefore, secondary structure can deter the protective function of this protein, leading to site-specific trinucleotide expansions.

Images

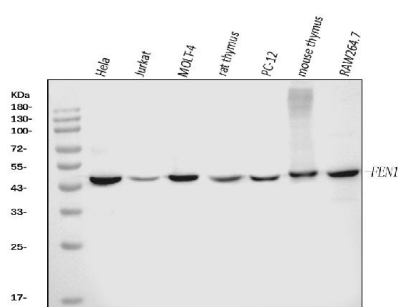


Figure 1. Western blot analysis of FEN1 using anti-FEN1 antibody (M01484-3).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,
Lane 2: human Jurkat whole cell lysates,
Lane 3: human MOLT-4 whole cell lysates,
Lane 4: rat thymus tissue lysates,
Lane 5: rat PC-12 whole cell lysates,
Lane 6: mouse thymus tissue lysates,
Lane 6: mouse RAW264.7 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-FEN1 antigen affinity purified monoclonal antibody

(Catalog # M01484-3) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for FEN1 at approximately 48 kDa. The expected band size for FEN1 is at 48 kDa.

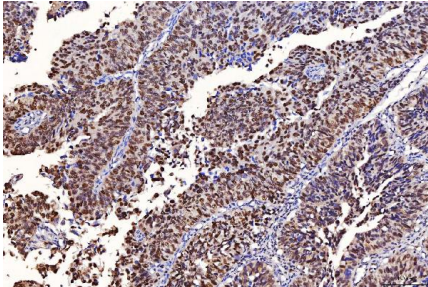


Figure 2. IHC analysis of FEN1 using anti-FEN1 antibody (M01484-3).

FEN1 was detected in a paraffin-embedded section of human bladder epithelial carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-FEN1 Antibody (M01484-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

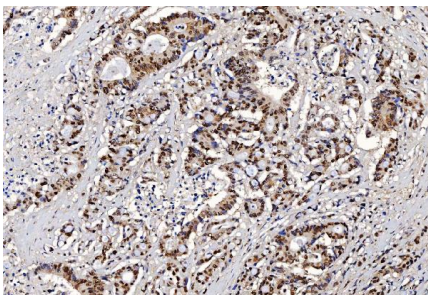


Figure 3. IHC analysis of FEN1 using anti-FEN1 antibody (M01484-3).

FEN1 was detected in a paraffin-embedded section of human colorectal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-FEN1 Antibody (M01484-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

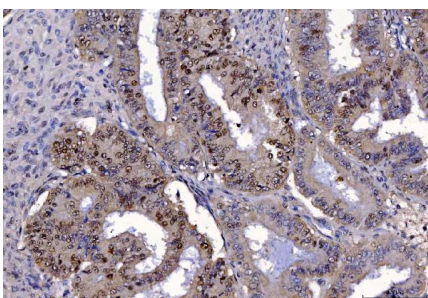
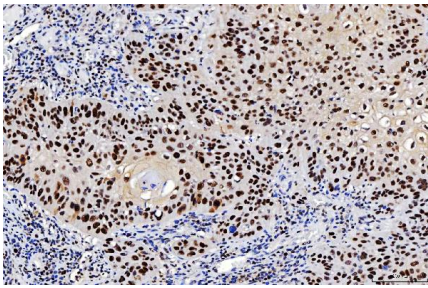


Figure 4. IHC analysis of FEN1 using anti-FEN1 antibody (M01484-3).

FEN1 was detected in a paraffin-embedded section of human endometrial cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-FEN1 Antibody (M01484-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

Figure 5. IHC analysis of FEN1 using anti-FEN1 antibody (M01484-3).

FEN1 was detected in a paraffin-embedded section of human laryngeal squamous cell carcinomas tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was



then incubated with 2 µg/ml mouse anti-FEN1 Antibody (M01484-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

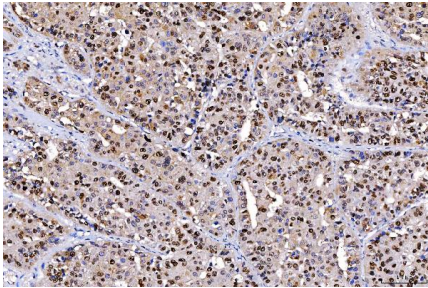


Figure 6. IHC analysis of FEN1 using anti-FEN1 antibody (M01484-3). FEN1 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-FEN1 Antibody (M01484-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

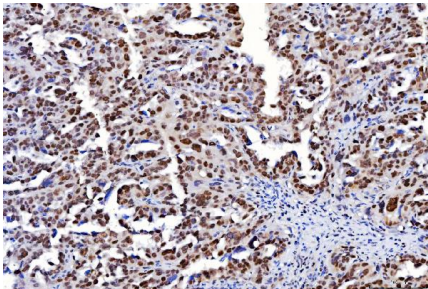


Figure 7. IHC analysis of FEN1 using anti-FEN1 antibody (M01484-3). FEN1 was detected in a paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-FEN1 Antibody (M01484-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

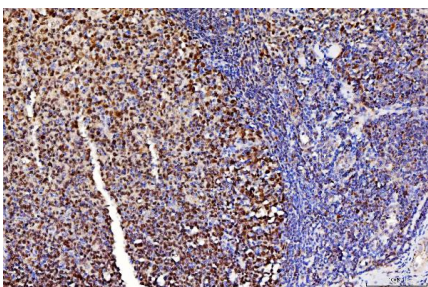


Figure 8. IHC analysis of FEN1 using anti-FEN1 antibody (M01484-3). FEN1 was detected in a paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-FEN1 Antibody (M01484-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

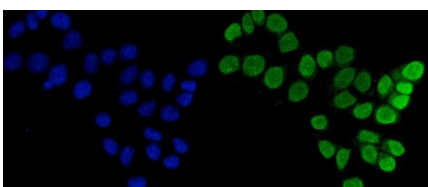


Figure 9. IF analysis of FEN1 using anti-FEN1 antibody (M01484-3). FEN1 was detected in an immunocytochemical section of MCF-7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-FEN1 Antibody (M01484-3) overnight at 4°C. DyLight®488

Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

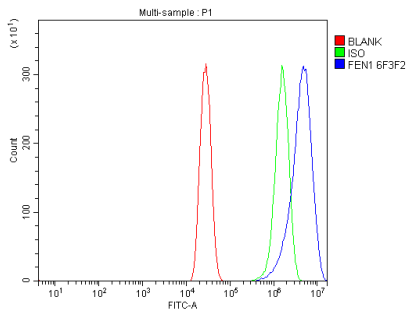


Figure 10. Flow Cytometry analysis of A431 cells using anti-FEN1 antibody (M01484-3). Overlay histogram showing A431 cells stained with M01484-3 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-FEN1 Antibody (M01484-3, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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