

TREVIGEN[®] Product Data

For Research Use Only. Not For Use In Diagnostic Procedures.

Anti-human XPF Antibody (clone 218)

Catalog#: 4431-MC-100

Size: 100 µg

Background: Mammalian XPF (from xeroderma pigmentosum factor F and also called ERCC4) associates with ERCC1 (Excision Repair Cross Complementing protein 1) to form a heterodimeric structure-specific DNA nuclease that functions in the nucleotide excision repair (NER) pathway. The ERCC1-XPF complex cleaves a damaged DNA strand on the 5' side of an opened "bubble" intermediate formed around the lesion. In addition, ERCC1-XPF appears to play a role in repair of interstrand DNA cross-link repair and has recently been demonstrated to cleave DNA on both sides of a psoralen cross-link.

Physical State: This antibody is provided as purified immunoglobulin from mouse ascites in 1X PBS containing 0.01% sodium azide.

Immunogen: Full length recombinant human XPF.

Ig Class: IgG_{2ax}

Specificity: Anti-XPF (clone 218) recognizes full length human XPF and specifically binds in the c-terminus region (amino acids 854-916). This antibody will not recognize proteolytic degradation fragments.

Storage: Store at 4°C. For extended storage, freeze at -20°C in working aliquots in a manual defrost freezer to avoid repeated freeze-thaws.

Applications: Western analysis. For western blots, an antibody dilution of 1:500-1:1000 is recommended. Empirical testing will be required for optimal results.

References:

1. McCutchen-Maloney, S.L., C.A. Gianecchini, M.H. Hwang and M. P. Thelen. 1999. Domain Mapping of the DNA Binding, Endonuclease, and ERCC1 binding properties of the human DNA repair protein XPF. *Biochemistry* **38**:9417-9425.
2. Brookman K.W., J.E. Lamerdin, M.P. Thelen, M.H. Hwang, J.T. Reardon, A. Sancar, Z-Q Zhou, C.A. Walter, C.N. Parris and L.H. Thompson. 1996. ERCC4 (XPF) encodes a human nucleotide excision repair protein with eukaryotic recombination homologs. *Mol Cell Bio* **16**:6553-6562.

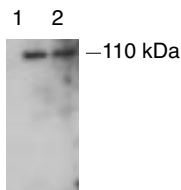


Figure 1. Immunoblot of whole cell extracts of 1) healthy Jurkat human cell line or 2) Jurkat treated with 45 J/m² of UltraViolet light at 254 nm. Samples were electrophoresed on a 12% Tris-Glycine gel and blotted onto a PVDF membrane. The 110 kDa band was detected after incubation with Trevigen's anti-XPF 218 diluted 1:1000, followed by anti-mouse-HRP and chemiluminescence.

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Suggested Preparation for Whole Cell Lysates:

Cells are harvested by cold centrifugation at 250 x g and washed with cold PBS. Cell pellets are then lysed in 2X cold RIPA Buffer (100 mM Tris-Cl, pH 7.4, 2% NP-40, 0.50 % Na-deoxycholate (DOC), 0.2 % SDS, 300 mM NaCl, 4 mM EDTA, 2 mM PMSF and protease inhibitors) at 1 x 10⁷ cells per ml and left on ice for 15 minutes. The extracts are centrifuged for 5 minutes at 500 X g, at 4°C, to remove debris. After addition of equal volume of 2X loading buffer, the extracts are heated at 95°C for 10 minutes and electrophoresed on a 12% Tris-Glycine SDS-PAGE gel.

Note: on figure 1, the equivalent of 1.25 X 10⁵ cells were loaded per well. Freeze-thawing of extracts is not recommended.

Procedure for Immunoblotting using Peroxidase Detection:

Transfer buffer: 12 mM Tris base, 96 mM Glycine, and 20% Methanol.

Blocking solution: 5% (w/v) nonfat dry milk in PBS.

Antibody solution: 5% (w/v) nonfat dry milk, 0.05% Tween in PBS.

Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1/2 hour at room temperature in blocking solution.

Incubate the membrane at room temperature for 2-3 hours or overnight at 4°C in antibody solution containing a 1:500-1:1000 dilution of anti-XPF (clone 218) monoclonal antibody. Empirical determination of primary antibody concentration will be required for optimal results.

Wash the membrane at room temperature for 15 minutes with 3 changes of 0.05% Tween in PBS. Changing the membrane containers often reduces background.

Incubate the membrane at room temperature for 1 hour in antibody solution containing anti-mouse conjugated to horseradish peroxidase. Empirical determination of secondary antibody concentration will be required for optimal results.

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

Develop peroxidase reaction using e.g. chemiluminescence (Trevigen's PeroxyGlow A, Cat# 4855-20-13, and PeroxyGlow B, Cat# 4855-20-14).

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