

TREVIGEN® Product Data

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

Anti-Human XRCC1 Antibody (clone 144)

Catalog#: 4421-MC-100

Size: 100 µg

Background: XRCC1 (X-ray repair cross complementing group 1) is involved in multiple DNA repair pathways and is required for DNA single-strand break repair. In human cells, XRCC1 interacts with DNA polymerase β, DNA ligase III, and polynucleotide kinase. These four proteins coassociate and repair single-strand breaks typical of those induced by radiation and reactive oxygen species. XRCC1 also associates with autoribosylated-PARP-1 at the sites of DNA breaks and stimulates the phosphatase and kinase activities of polynucleotide kinase to accelerates the rate of repair.

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

Immunogen: Full length polypeptide of human XRCC1.

Ig Class: IgG_{2ax}

Specificity: Anti-XRCC1 is specific for human XRCC1.

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

Applications: Western analysis. For western blots, an antibody dilution of 1:1250 is recommended. Empirical testing will be required for optimal results.

References: Shen, M.R., M.Z. Zdzienicka, H. Mohrenweiser, L.H. Thompson and M.P. Thelen. 1998 Mutations in hamster single-strand break repair gene XRCC1 causing defective DNA repair. *Nucleic Acids Res.* 26:1032-1037.

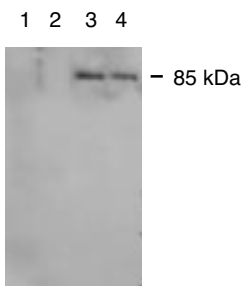


Figure 1. Immunoblot of whole cell extracts of 1) Healthy WEHI 7.1 mouse lymphoma and 2) treated with 45 J/m² of UltraViolet light at 254 nm. 3) and 4) healthy and UV treated Jurkat human cell line. Samples were electrophoresed on a 12% Tris-Glycine gel and blotted onto a PVDF membrane. Overnight incubation with anti-XRCC1 (clone 144) diluted 1:1250, was followed by anti-mouse-HRP and chemiluminescence. The 85 kDa XRCC1 was detected in the untreated and UV treated human samples.

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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 solubilized in cold 2X 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) 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 in a boiling water bath 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.

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 overnight at 4°C in antibody solution containing a 1:1250 dilution of anti-XRCC1 (clone 144) 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).

Anti-XRCC1 (144)
Catalog #: 4421-MC-100
Lot#:4474J1
Storage: 4°C

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