abcam

Product datasheet

Anti-XLF antibody ab33499

KO VALIDATED

3 Abreviews 10 References 6 Images

Overview

Product name	Anti-XLF antibody
Description	Rabbit polyclonal to XLF
Host species	Rabbit
Tested applications	**Suitable for:** IP, IHC-P, ICC/IF, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Dog
Immunogen	Synthetic peptide corresponding to Human XLF aa 250 to the C-terminus (C terminal). (Peptide available as ab27783)
Positive control	HeLa whole cell or nuclear extract, A431 and Jurkat whole cell extracts.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS. pH 7.4
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab33499** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

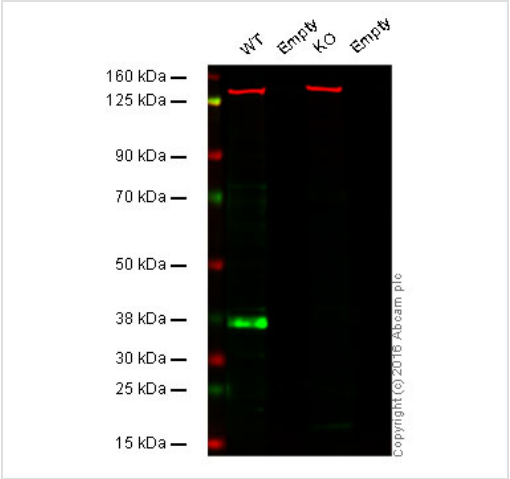
Application	Abreviews	Notes
IP		Use at an assay dependent concentration.

Application	Abreviews	Notes
IHC-P		1/125. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF	★★★☆☆	Use a concentration of 1 µg/ml.
WB	★★★★★	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 37 kDa (predicted molecular weight: 35 kDa).

Target

Function	DNA repair protein involved in DNA nonhomologous end joining (NHEJ) required for double-strand break (DSB) repair and V(D)J recombination. May serve as a bridge between XRCC4 and the other NHEJ factors located at DNA ends, or may participate in reconfiguration of the end bound NHEJ factors to allow XRCC4 access to the DNA termini. It may act in concert with XRCC6/XRCC5 (Ku) to stimulate XRCC4-mediated joining of blunt ends and several types of mismatched ends that are noncomplementary or partially complementary.
Tissue specificity	Ubiquitously expressed.
Involvement in disease	Defects in NHEJ1 are the cause of severe combined immunodeficiency due to NHEJ1 deficiency (NHEJ1-SCID) [MIM:611291]; also known as autosomal recessive T cell-negative, B cell-negative, NK cell-positive, severe combined immunodeficiency with microcephaly, growth retardation and sensitivity to ionizing radiation or NHEJ1 syndrome. SCID refers to a genetically and clinically heterogeneous group of rare congenital disorders characterized by impairment of both humoral and cell-mediated immunity, leukopenia and low or absent antibody levels. Patients with SCID present in infancy with recurrent, persistent infections by opportunistic organisms. The common characteristic of all types of SCID is absence of T-cell-mediated cellular immunity due to a defect in T-cell development. NHEJ1-SCID is characterized by a profound T- and B-lymphocytopenia associated with increased cellular sensitivity to ionizing radiation, microcephaly and growth retardation. Some patients may manifest SCID with sensitivity to ionizing radiation without microcephaly and mild growth retardation, probably due to hypomorphic NHEJ1 mutations. Note=A chromosomal aberration involving NHEJ1 is found in a patient with polymicrogyria. Translocation t(2;7)(q35;p22).
Sequence similarities	Belongs to the XLF family.
Cellular localization	Nucleus.

Images

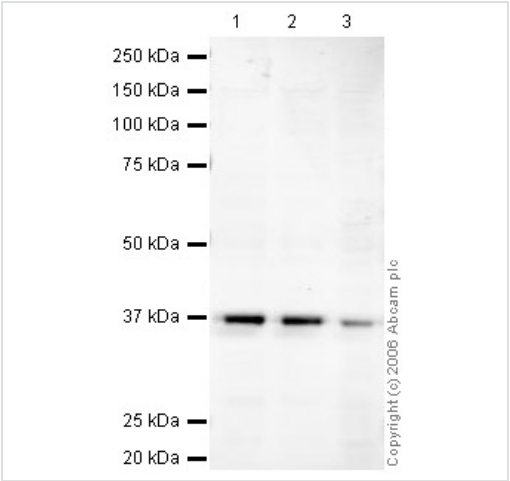


Western blot - Anti-XLF antibody (ab33499)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: XLF knockout HAP1 cell lysate (20 µg)

Lanes 1 - 2: Merged signal (red and green). Green – ab33499 observed at 38 kDa. Red - loading control, ab18058, observed at 124 kDa.

ab33499 was shown to specifically react with XLF when XLF knockout samples were used. Wild-type and XLF knockout samples were subjected to SDS-PAGE. ab33499 and ab18058 (loading control to Vinculin) were diluted at 1 µg/mL and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-XLF antibody (ab33499)

All lanes : Anti-XLF antibody (ab33499) at 1 µg/ml

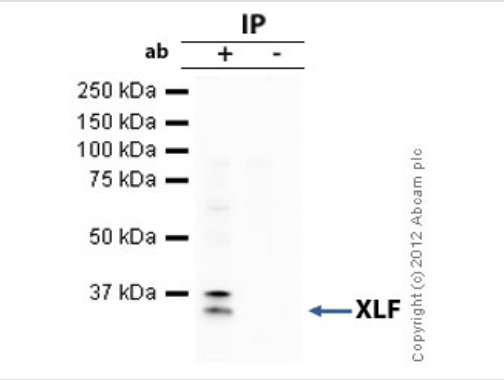
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : Jurkat whole cell lysate (ab7899)
Lane 3 : A431 whole cell lysate (ab7909)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG (Alexa Fluor® 680) at 1/10000 dilution

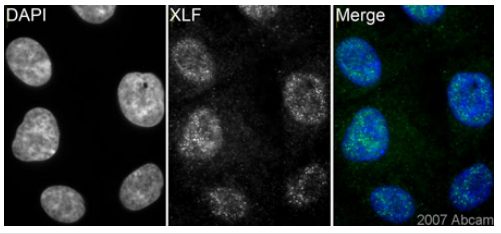
Performed under reducing conditions.

Predicted band size: 35 kDa
Observed band size: 37 kDa



Immunoprecipitation - Anti-XLF antibody (ab33499)

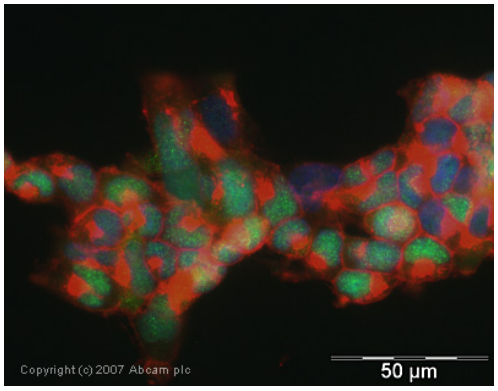
XLF was immunoprecipitated using 0.5mg A431 whole cell extract, 5µg of Rabbit polyclonal to XLF and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, A431 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{O}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab33499.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).
Band: Bands: 35kDa: XLF; non specific - 37kDa: We are unsure as to the identity of this extra band.



Immunocytochemistry/ Immunofluorescence - Anti-XLF antibody (ab33499)

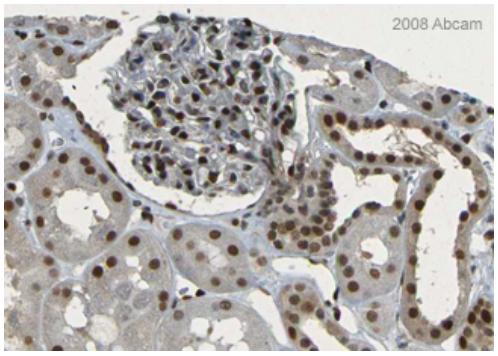
This image is courtesy of an Abreview submitted by Dr Kirk McManus

ab33499 (1/200) staining XLF in assynchronous, bleomycin treated, human RPE-1 cells (green). Cells were fixed in paraformaldehyde, permeabilised in 0.5% Triton X100/PBS and counterstained with DAPI in order to highlight the nucleus (blue). Please refer to abreview for further experimental details.



Immunocytochemistry/ Immunofluorescence - Anti-XLF antibody (ab33499)

ICC/IF image of ab33499 stained human HEK 293 cells. The cells were PFA fixed (10 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab33499, 1µg/ml) for 1h at room temperature. 1%BSA / 10% normal serum / 0.3M glycine was used to quench autofluorescence and block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-XLF antibody (ab33499)

Image courtesy of Human Protein Atlas

ab33499 staining XLF in human kidney. Paraffin embedded human kidney tissue was incubated with ab33499 (1/125 dilution) for 30 mins at room temperature. Antigen retrieval was performed by heat induction in citrate buffer pH 6.

ab33499 was tested in a tissue microarray (TMA) containing a wide range of normal and cancer tissues as well as a cell microarray consisting of a range of commonly used, well characterised human cell lines.

Further results for this antibody can be found at www.proteinatlas.org

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise,

please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors