

Product datasheet

Human ANXA6 (Annexin-6) knockout HeLa cell lysate ab257351

5 Images

Overview

Product name	Human ANXA6 (Annexin-6) knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon7 and 5 bp deletion in exon7.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found [here](#). Please refer to our lysis protocol for further details on how our lysates are prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

[See here for more information on knockout cell lysates.](#)

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It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

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Tested applications **Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab260969 - Human ANXA6 knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial
Disease Adenocarcinoma
Gender Female
STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 WWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

Target

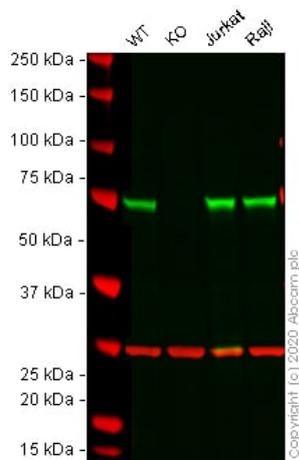
Function May associate with CD21. May regulate the release of Ca(2+) from intracellular stores.
Sequence similarities Belongs to the annexin family.
Contains 8 annexin repeats.
Domain A pair of annexin repeats may form one binding site for calcium and phospholipid.
Post-translational modifications Phosphorylated in response to growth factor stimulation.
Cellular localization Cytoplasm. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257351 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
WB		Use at an assay dependent concentration. Predicted molecular weight: 76 kDa.

Images



Western blot - Human ANXA6 (Annexin-6) knockout HeLa cell lysate (ab257351)

Lane 1: Wild-type HeLa cell lysate 20 ug

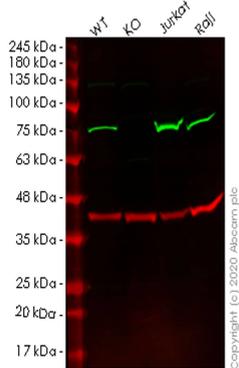
Lane 2: ANXA6 knockout HeLa cell lysate 20 ug

Lane 3: Jurkat cell lysate 20 ug

Lane 4: Raji cell lysate 20 ug

Lanes 1 - 4: Merged signal (red and green). Green - **ab199422** observed at 75 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab199422 was shown to react with Annexin-6/ANXA6 in wild-type HeLa cells in western blot with loss of signal observed in ANXA6 knockout cell line **ab265677** (ANXA6 knockout cell lysate ab257351). Wild-type and ANXA6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween[®]) before incubation with **ab199422** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human ANXA6 knockout HeLa cell lysate (ab257351)

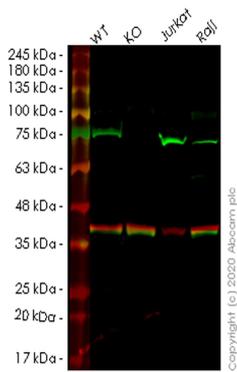
Lane 1: Wild-type HeLa cell lysate (20 ug)

Lane 2: ANXA6 knockout HeLa cell lysate (20 ug)

Lane 3: Jurkat cell lysate (20 ug)

Lane 4: Raji cell lysate (20 ug)

ab201023 was shown to specifically react with Annexin-6/ANXA6 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265677** (knockout cell lysate ab257351) was used. Wild-type and Annexin-6/ANXA6 knockout samples were subjected to SDS-PAGE. **ab201023** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human ANXA6 knockout HeLa cell lysate (ab257351)

Lane 1: Wild-type HeLa cell lysate (20 ug)

Lane 2: ANXA6 knockout HeLa cell lysate (20 ug)

Lane 3: Jurkat cell lysate (20 ug)

Lane 4: Raji cell lysate (20 ug)

ab201024 was shown to specifically react with Annexin-6/ANXA6 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265677** (knockout cell lysate ab257351) was used. Wild-type and Annexin-6/ANXA6 knockout samples were subjected to SDS-PAGE. **ab201024** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.

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Mut  CACATGTTCTTTGTCTTTCTGCCAAGGGAA----GAGGAGGATGACGTAGTGAGCGAGG
      |||
WT   CACATGTTCTTTGTCTTTCTGCCAAGGGAAACCAGGGAGGAGGATGACGTAGTGAGCGAGG
  
```

Sanger Sequencing - Human ANXA6 knockout HeLa cell lysate (ab257351)

Allele-1: 5 bp deletion in exon7

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Mut  CACATGTTCTTTGTCTTTCTGCCAAGGGAAACCAGGGAGGAGGATGACGTAGTGAGCGAGG
      |||
WT   CACATGTTCTTTGTCTTTCTGCCAAGGGAA  CCAGGGAGGAGGATGACGTAGTGAGCGAGG
  
```

Sanger Sequencing - Human ANXA6 knockout HeLa cell lysate (ab257351)

Allele-2: 1 bp insertion in exon7

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