abcam

Product datasheet

# Human TUBB3 (beta III Tubulin) knockout HeLa cell lysate ab263857

3 Images

## Overview

| | |
|---|---|
| **Product name** | Human TUBB3 (beta III Tubulin) 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, Homozygous: 1 bp insertion in exon 4. |
| **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.<br>**Usage of SDS sample buffer is not recommended with these lyophilized lysates.* |
| **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.<br><br>**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.<br><br>Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.<br>**See here for more information on knockout cell lysates.**<br><br>Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.<br>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.<br><br>This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**. |
| **Tested applications** | **Suitable for:** WB |

## Properties

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

| Components | 1 kit |
|---|---|
| ab255466 - Human TUBB3 knockout HeLa cell lysate | 1 x 100µg |
| ab255552 - 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 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10 |

## Target

| | |
|---|---|
| **Function** | Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain. TUBB3 plays a critical role in proper axon guidance and mantainance. |
| **Tissue specificity** | Expression is primarily restricted to central and peripheral nervous system. |
| **Involvement in disease** | Defects in TUBB3 are the cause of congenital fibrosis of extraocular muscles type 3A (CFEOM3A) [MIM:600638]. A congenital ocular motility disorder marked by restrictive ophthalmoplegia affecting extraocular muscles innervated by the oculomotor and/or trochlear nerves. It is clinically characterized by anchoring of the eyes in downward gaze, ptosis, and backward tilt of the head. Congenital fibrosis of extraocular muscles type 3 presents as a non-progressive, autosomal dominant disorder with variable expression. Patients may be bilaterally or unilaterally affected, and their oculo-motility defects range from complete ophthalmoplegia (with the eyes fixed in a hypo- and exotropic position), to mild asymptomatic restrictions of ocular movement. Ptosis, refractive error, amblyopia, and compensatory head positions are associated with the more severe forms of the disorder. In some cases the ocular phenotype is accompanied by additional features including developmental delay, corpus callosum agenesis, basal ganglia dysmorphism, facial weakness, polyneuropathy. |
| **Sequence similarities** | Belongs to the tubulin family. |
| **Domain** | The highly acidic C-terminal region may bind cations such as calcium. |
| **Post-translational modifications** | Some glutamate residues at the C-terminus are polyglutamylated. This modification occurs exclusively on glutamate residues and results in polyglutamate chains on the gamma-carboxyl group. Also monoglycylated but not polyglycylated due to the absence of functional TTLL10 in human. Monoglycylation is mainly limited to tubulin incorporated into axonemes (cilia and flagella) whereas glutamylation is prevalent in neuronal cells, centrioles, axonemes, and the mitotic spindle. Both modifications can coexist on the same protein on adjacent residues, and lowering glycylation levels increases polyglutamylation, and reciprocally. The precise function of such modifications is still unclear but they regulate the assembly and dynamics of axonemal microtubules. |
| **Cellular localization** | Cytoplasm > cytoskeleton. |

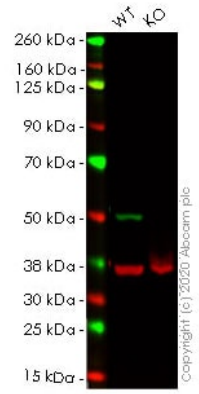
## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab263857 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. |

## Images



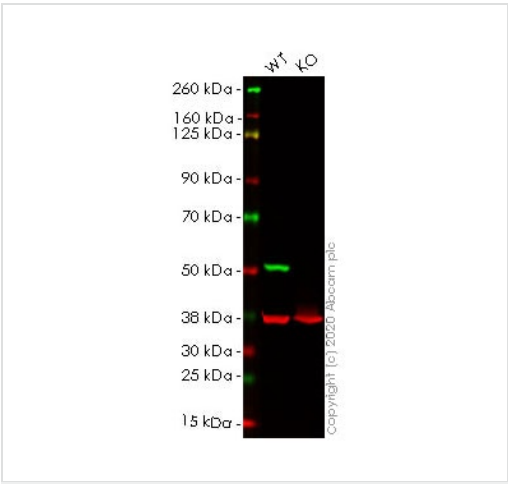

Western blot - Human TUBB3 (beta III Tubulin) knockout HeLa cell lysate (ab263857)

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

**Lane 2:** TUBB3 knockout HeLa cell lysate (20µg)

**Lanes 1- 2:** Merged signal (red and green). Green - **ab215037** observed at 50 kDa. Red - loading control **ab8245** observed at 37 kDa.

**ab215037** Recombinant Anti-beta III Tubulin antibody [EPR19591] was shown to specifically react with TUBB3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab255358** (knockout cell lysate ab263857) was used. Wild-type and TUBB3 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab215037** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 2000 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 TUBB3 (beta III Tubulin) knockout HeLa cell lysate (ab263857)

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

**Lane 2:** TUBB3 knockout HeLa cell lysate (20µg)

**Lanes 1- 2:** Merged signal (red and green). Green - **ab52623** observed at 50 kDa. Red - loading control **ab8245** observed at 37 kDa.

**ab52623** Recombinant Anti-beta III Tubulin antibody [EP1569Y] was shown to specifically react with TUBB3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab255358** (knockout cell lysate ab263857) was used. Wild-type and TUBB3 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab52623** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4˚C 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.


Sanger Sequencing - Human TUBB3 knockout HeLa cell lysate (ab263857)

Homozygous: 1 bp insertion in exon 4

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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