

Properties

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

Components	1 kit
ab263502 - Human TUBB3 knockout HCT116 cell lysate	1 x 100µg
ab255555 - Human wild-type HCT116 cell lysate	1 x 100µg

Cell type epithelial

Disease Carcinoma

STR Analysis Amelogenin X D5S818: 10, 11 D13S317: 10, 12 D7S820: 11, 12 D16S539: 11, 13 vWA: 17, 22 TH01: 8,9 TPOX: 8, 9 CSF1PO: 7, 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 maintenance.

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 TLL10 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 polyglutamylated, 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

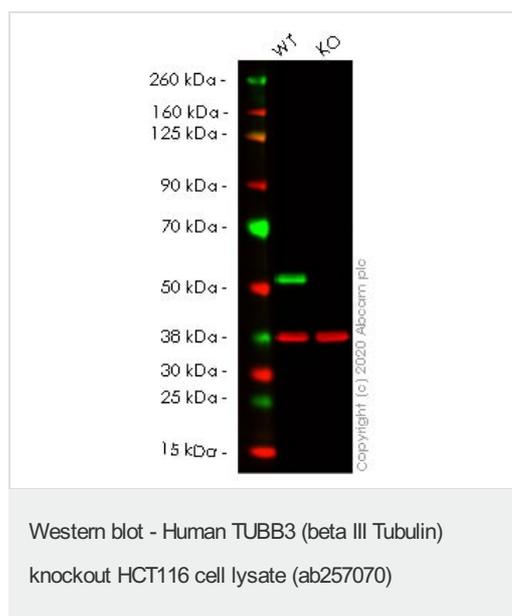
The Abpromise guarantee

Our [Abpromise guarantee](#) covers the use of ab257070 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: 50 kDa.

Images

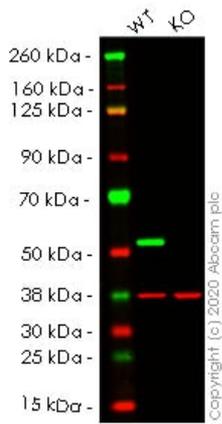


Lane 1: Wild-type HCT116 cell lysate (20µg)

Lane 2: TUBB3 knockout HCT116 cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - [ab52623](#) observed at 52 kDa. Red - loading control [ab8245](#) observed at 37 kDa.

[ab52623](#) Anti-beta III Tubulin antibody [EP1569Y] was shown to specifically react with beta III Tubulin in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line [ab266900](#) (knockout cell lysate ab257070) was used. Wild-type and beta III Tubulin 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 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 HCT116 cell lysate (ab257070)

Lane 1: Wild-type HCT116 cell lysate (20µg)

Lane 2: TUBB3 knockout HCT116 cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - [ab215037](#) observed at 52 kDa. Red - loading control [ab8245](#) observed at 37 kDa.

[ab215037](#) Anti-beta III Tubulin antibody [EPR19591] was shown to specifically react with beta III Tubulin in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line [ab266900](#) (knockout cell lysate ab257070) was used. Wild-type and beta III Tubulin 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 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  CTCCTTACCCCTCTTCTCCCTGTACAGGTCAAGAGTGGGGCCGGCAACAACCTGGGCCAAGG
      |||
WT   CTCCTTACCCCTCTTCTCCCTGTACAGGTCAAGAGTGGGGCCGGCAACAACCTGGGCCAAGG
  
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Sanger Sequencing - Human TUBB3 knockout HCT116 cell lysate (ab257070)

Homozygous: 1 bp insertion in exon4

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

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