# abcam

### Product datasheet

## Human YWHAG (14-3-3 gamma) knockout HEK-293T cell line ab266734

#### 3 Images

Overview

| Product name         | Human YWHAG (14-3-3 gamma) knockout HEK-293T cell line  |  |  |
|----------------------|---|--|--|
| Parental Cell Line   | HEK293T   |  |  |
| Organism             | Human   |  |  |
| Mutation description | Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 1  |  |  |
| Passage number       | <20   |  |  |
| Knockout validation  | Sanger Sequencing, Western Blot (WB)  |  |  |
| Tested applications  | Suitable for: WB  |  |  |
| Biosafety level      | 2   |  |  |
| General notes        | <b>Recommended control:</b> Human wild-type HEK293T cell line ( <u>ab255449</u> ). Please note a wild-<br>type cell line is not automatically included with a knockout cell line order, if required please add<br>recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.  |  |  |
|                      | <b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.  |  |  |
|                      | Culture medium: DMEM (High Glucose) + 10% FBS   |  |  |
|                      | <b>Initial handling guidelines:</b> Upon arrival, the vial should be stored in liquid nitrogen vapor phas and not at -80°C. Storage at -80°C may result in loss of viability.   |  |  |
|                      | <ol> <li>Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifug tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution.</li> <li>Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of 2x10<sup>4</sup> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> <li>Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> </ol> |  |  |
|                      | Subculture guidelines:  |  |  |
|                      | All seeding densities should be based on cell counts gained by established methods.<br>A guide seeding density of 2x10 <sup>4</sup> cells/cm <sup>2</sup> is recommended.   |  |  |

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence. 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**.

We will provide viable cells that proliferate on revival.

#### Properties

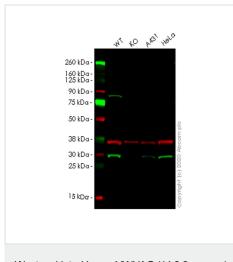
| Number of cells                  | 1 x 10 <sup>6</sup> cells/vial, 1 mL   |  |  |
|----------------------------------|--|--|--|
| Adherent /Suspension             | Adherent   |  |  |
| Tissue                           | Kidney   |  |  |
| Cell type                        | epithelial   |  |  |
| STR Analysis                     | Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12   |  |  |
| Mycoplasma free                  | Yes  |  |  |
| Storage instructions             | Shipped on Dry Ice. Store in liquid nitrogen.  |  |  |
| Storage buffer                   | Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether   |  |  |
|                                  |  |  |  |
| Target                           |  |  |  |
| Function                         | Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner. |  |  |
| Tissue specificity               | Highly expressed in brain, skeletal muscle, and heart.   |  |  |
| Sequence similarities            | Belongs to the 14-3-3 family.  |  |  |
| Post-translational modifications | Phosphorylated by various PKC isozymes.  |  |  |
| Cellular localization            | Cytoplasm.   |  |  |
|                                  |  |  |  |

#### **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab266734 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: 28 kDa. |



Western blot - Human YWHAG (14-3-3 gamma) knockout HEK293T cell line (ab266734) All lanes : Anti-14-3-3 gamma/YWHAG antibody [EPR9654(B)] (ab137048) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate Lane 2 : YWHAG knockout HEK293T cell lysate Lane 3 : A431 cell lysate Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Predicted band size: 28 kDa Observed band size: 28 kDa

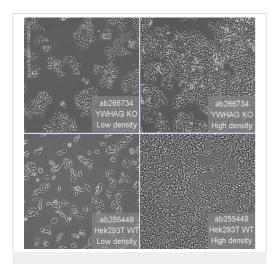
Lanes 1-4: Merged signal (red and green). Green - <u>ab137048</u> observed at 28 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

<u>ab137048</u> Anti-14-3-3 gamma/YWHAG antibody [EPR9654(B)] was shown to specifically react with 14-3-3 gamma/YWHAG in wildtype HEK293T cells. Loss of signal was observed when knockout cell line ab266734 (knockout cell lysate <u>ab257805</u>) was used. Wildtype and 14-3-3 gamma/YWHAG knockout samples were subjected to SDS-PAGE. <u>ab137048</u> and Anti-GAPDH antibody [6C5] -Loading Control (<u>ab8245</u>) 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<sup>®</sup> 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Homozygous: 1 bp insertion in exon1



Sanger Sequencing - Human YWHAG knockout HEK293T cell line (ab266734)



Human YWHAG (14-3-3 gamma) knockout HEK293T cell line (ab266734)

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

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