

PRODUCT INFORMATION

Product name : ID3 antibody

Product type : Primary antibodies

Description : Rabbit polyclonal to ID3

Immunogen : 3 synthetic peptides (human) conjugated to KLH

Reacts with : Hu, Ms

Tested applications : ELISA, WB and IF

GENE INFORMATION

Gene Symbol : ID3

Gene Name : inhibitor of DNA binding 3, dominant negative helix-loop-helix protein

Ensembl ID : ENSG00000117318

Entrez GeneID : 3399

GenBank Accession number : X69111

Omim ID : 600277

Swiss-Prot : Q02535

Molecular weight of ID3 : 13 kDa

Function : ID (inhibitor of DNA binding) HLH proteins lack a basic DNA-binding domain but are able to form heterodimers with other HLH proteins, thereby inhibiting DNA binding. ID-3 inhibits the binding of E2A-containing protein complexes to muscle creatine kinase E-box enhancer. May inhibit other transcription factors.

Expected subcellular localization : Nucleus

Expected tissue specificity : Expressed abundantly in lung, kidney and adrenal gland, but not in adult brain.

Summary : Members of the ID family of helix-loop-helix (HLH) proteins lack a basic DNA-binding domain and inhibit transcription through formation of nonfunctional dimers that are incapable of binding to DNA. [supplied by OMIM]

APPLICATION NOTE

Recommended dilution :

- **ELISA:** Antibody specificity was verified by direct ELISA against the 3 immunogen peptides. A titer of 1/13000 has been determined. Appropriate specificity controls were run.
- **WB:** 1/5000.
- **IF:** 1/1000.

Optimal dilutions/concentration should be determined by the end user.

Raised in : Rabbit

Clonality : Polyclonal

Isotype : IgG

Purity : Purified antibody

Storage buffer : 0.5 X PBS, containing a final concentration of 50% Glycerol, 0.1% BSA and 0.01% Thimerosal.

Form : Liquid

Storage instruction : Store at -20°C or -80°C. Avoid repeated freeze / thaw cycles

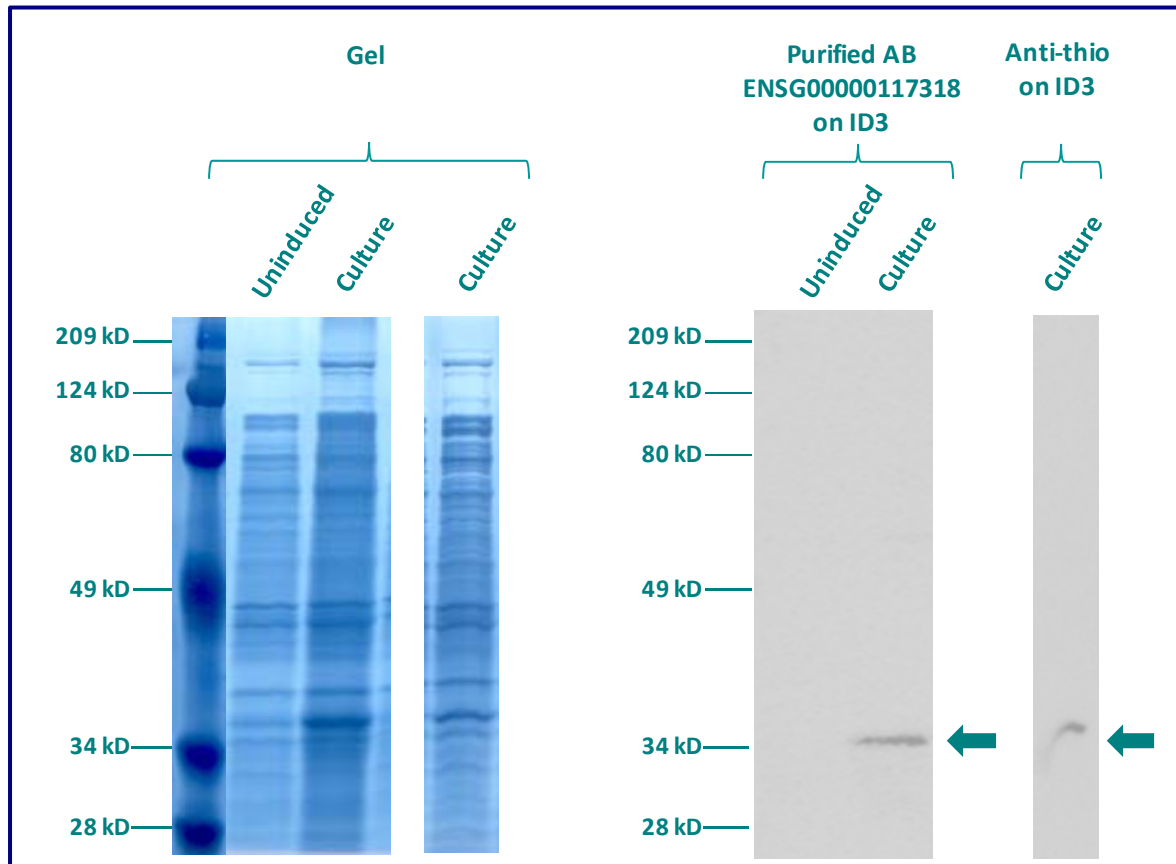
WESTERN BLOT ON RECOMBINANT PROTEIN

The purified antibody ENSG00000117318 has been tested at 1/5000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells).

An anti-thio (positive control) has been tested at 1/5000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells) as a positive control.

Plasmid name : pBAD-DEST49.

Molecular weight of ID3 : 27kDa (13kDa + another 14kDa for the tag).



Gel concentration: 10%

Blocking: in 5% non-fat milk-PBST solution

1st Antibody: The antibodies are diluted in blocking buffer.

- Dilute the purified antibody ENSG00000117318 at 1:5000
- Dilute the anti-thio at 1:5000

60 minutes of incubation

2nd Antibody: The antibody is diluted in blocking buffer.

- Dilute the anti-Rabbit IgG HRP conjugated at 1/10000
- 60 minutes of incubation

IMMUNOFLUORESCENCE ANALYSIS

Immunofluorescence analysis of DNA-binding protein inhibitor ID-3 (ID3) expression in 6 cells lines (HELA, 293T/17, Capan-2, SAOS-2, SH-SY5Y, Skin 3,44). The purified Antibody ENSG00000117318 has been tested at 1/5000.

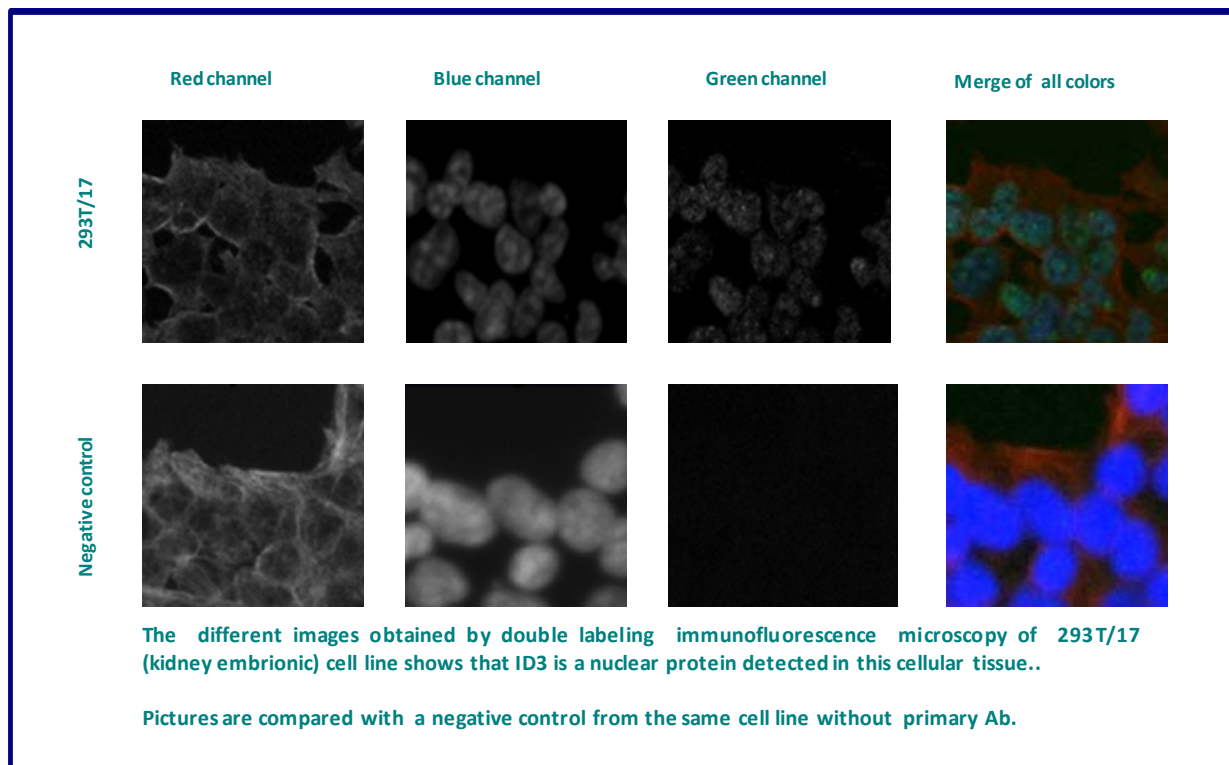
Red staining : cytoskeleton (microtubules/ α -tubuline)

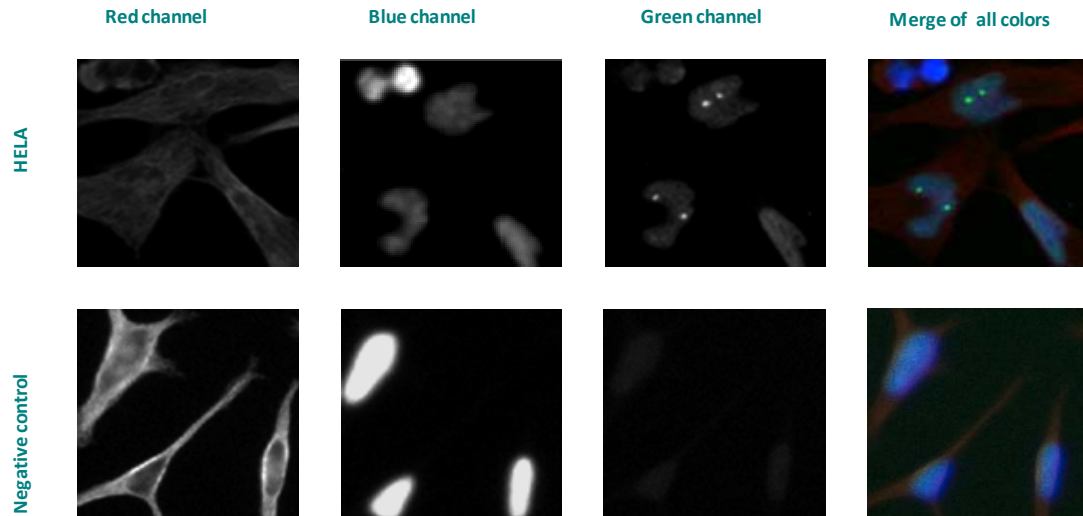
Blue staining : nucleus (Hoechst)

Green staining : anti- ID3 antibody (purified)

Expected subcellular location : Nucleus

Expected tissue specificity : Expressed abundantly in lung, kidney and adrenal gland, but not in adult brain.





The different images obtained by double labeling immunofluorescence microscopy of HELA (cervix adenocarcinoma) cell line shows that ID3 is a nuclear protein detected in this cellular tissue.

Pictures are compared with a negative control from the same cell line without primary Ab.

Remaining cell lines tested gave a positive result with a nuclear distribution.

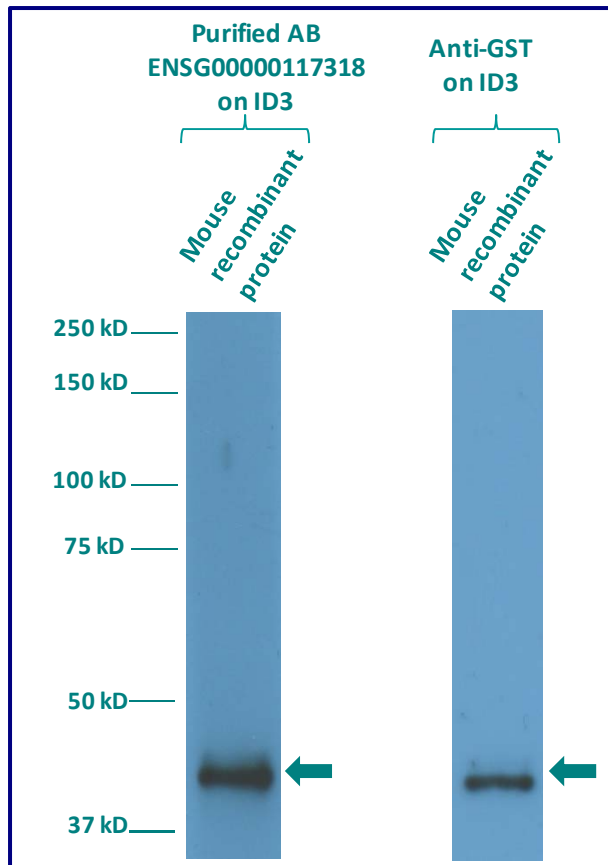
WESTERN BLOT ANALYSIS OF MOUSE RECOMBINANT PROTEIN ID3

The purified antibody ENSG00000117318 has been tested at 1/5000 on mouse recombinant protein.

An anti-GST has been tested as a positive control.

Plasmid name : pF3A-WG-GST.

Molecular weight of ID3 : 39kDa (13kDa + another 26kDa for the tag).



Gel concentration: 10%

Blocking: in 5% non-fat milk-PBST solution

1st Antibody: The antibodies are diluted in blocking buffer.

- Dilute the purified antibody ENSG00000117318 at 1:5000
- Dilute the anti-GST at 1:2000

60 minutes of incubation

2nd Antibody: The antibody is diluted in blocking buffer.

- Dilute the anti-Rabbit IgG HRP conjugated at 1/5000
- 60 minutes of incubation