PRODUCT INFORMATION

Product name：GRLF1 antibody

Product type： Primary antibodies

Description： Mouse monoclonal to GRLF1

Immunogen：1 synthetic peptide (human) conjugated to KLH

Reacts with： Hu, Ms

Tested applications：ELISA, WB & IF

GENE INFORMATION

Gene Symbol：GRLF1

Gene Name：Rho GTPase activating protein 35

Ensembl ID：ENSG00000160007

Entrez GeneID：2909

GenBank Accession number：M73077

Swiss-Prot：Q9NRY4

Molecular weight：170.5kDa

Function：Represses transcription of the glucocorticoid receptor by binding to the cis-acting regulatory sequence 5'-GAGAAAAGAAACTGGAGAAACTC-3'. May participate in the regulation of retinal development and degeneration. May transduce signals from p21-ras to the nucleus, acting via the ras GTPase-activating protein (GAP). May also act as a tumor suppressor.

Expected subcellular localization：Cytoplasm. Nucleus.

Summary：The human glucocorticoid receptor DNA binding factor, which associates with the promoter region of the glucocorticoid receptor gene (hGR gene), is a repressor of glucocorticoid receptor transcription. The amino acid sequence deduced from the cDNA sequences show the presence of three sequence motifs characteristic of a zinc finger and one motif suggestive of a leucine zipper in which 1 cysteine is found instead of all leucines. The GRLF1 enhances the homologous down-regulation of wild-type hGR gene expression. Biochemical analysis suggests that GRLF1 interaction is sequence specific and that transcriptional efficacy of GRLF1 is regulated through its interaction with specific sequence motif. The level of expression is regulated by glucocorticoids. [provided by RefSeq, Jul 2008]

APPLICATION NOTE

Recommended dilution：

- **ELISA: Antibody specificity was verified by direct ELISA against the 1 immunogen peptide. A titer of 8000 has been determined. Appropriate specificity controls were run.**
- **WB: Dilution 1/5000**
- **IF: Dilution 1/50**

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

Raised in：Mouse

Clonality：Monoclonal

Isotype：IgG

Purity：Purified Antibody

Concentration: 0.5mg/ml

Storage buffer： Containing a final concentration of PBS/glycerol (V/V), 0.1% BSA and 0.01% Thimerosal.

Form：Liquid

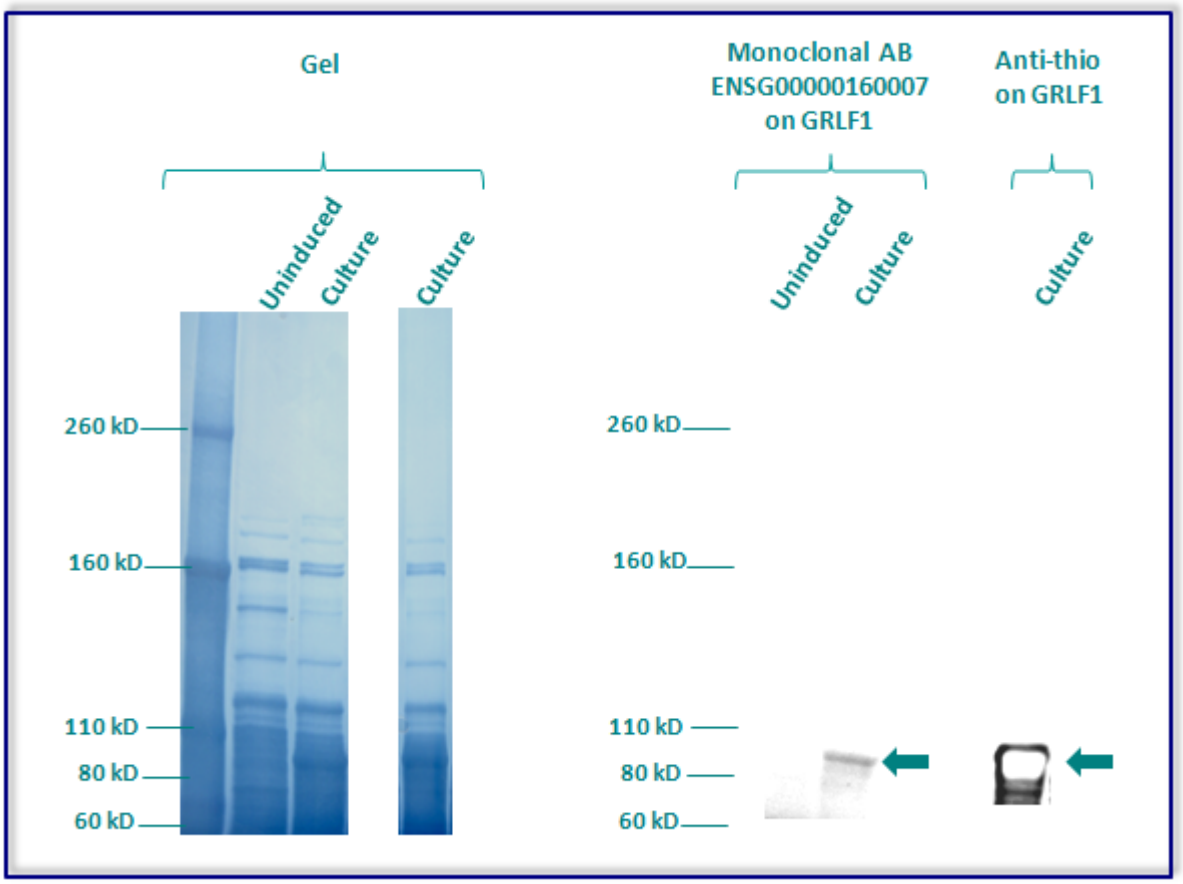
Storage instruction：Store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

WESTERN BLOT ON RECOMBINANT PROTEIN

The monoclonal purified antibody ENSG00000160007 has tested at 1/5000 on uninduced (negative control) and induced culture of E.coli (one shot Top10 competent cells).

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

Clone : 1G1D12, Isotype : G1; kappa
Plasmid name : pBAD-DEST49.
Molecular weight of GRLF1 : 100.3kDa (86.3kDa + another 14kDa for the tag).
Note: The size of the recombinant protein produced by the GRLF1 clone was 86.3kDa and not the 170.5kDa of the endogenous protein.



Gel concentration: 5%

Blocking: in 5% non-fat milk-PBST solution
1st Antibody: The antibodies are diluted in blocking buffer.

- **Dilute the purified antibody ENSG00000160007 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-Mouse IgG HRP conjugated at 1/10000**

60 minutes of incubation

IMMUNOFLUORESCENCE ANALYSIS

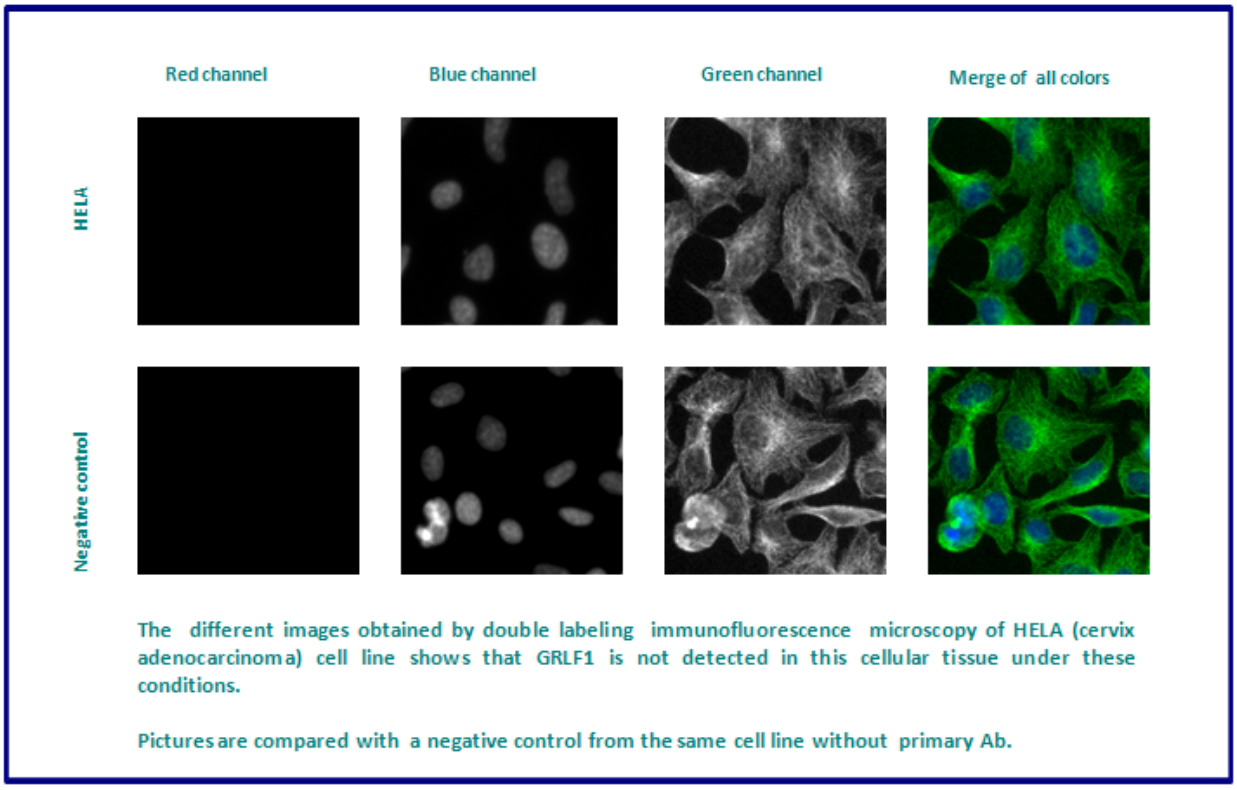
Immunofluorescence analysis of Rho GTPase-activating protein 35 (GRLF1) expression in 6 cells lines (HELA, 293T/17, Capan-2, SAOS-2, SH-SY5Y, Skin 3,44). The monoclonal antibody ENSG00000160007 has been tested at 1/50.

Green staining : cytoskeleton (microtubules/α-tubuline)

Blue staining : nucleus (Hoechst)

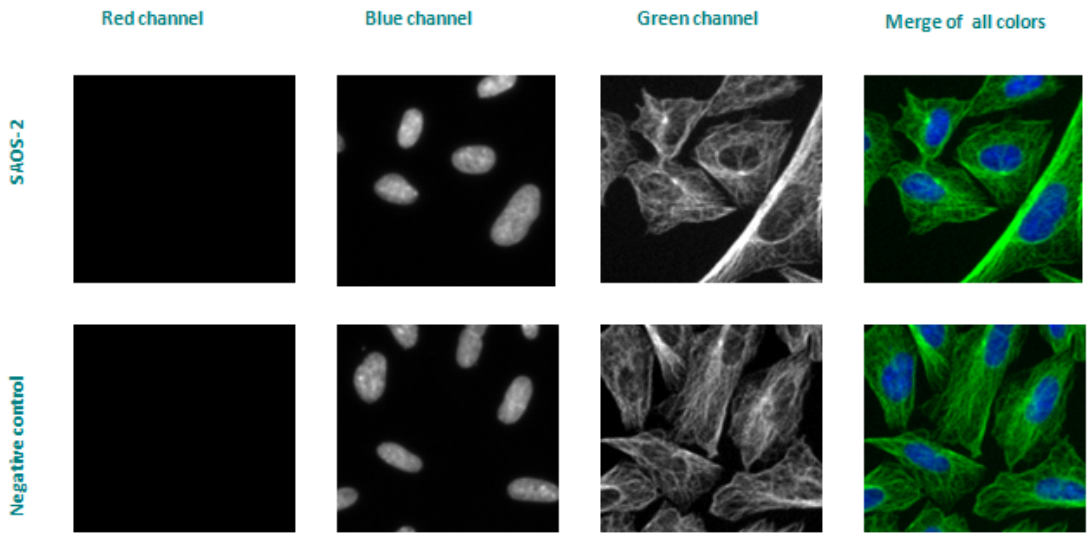
Red staining : anti- GRLF1 antibody (purified)

Expected subcellular location : Cytoplasm. Nucleus



The different images obtained by double labeling immunofluorescence microscopy of HELA (cervix adenocarcinoma) cell line shows that GRLF1 is not detected in this cellular tissue under these conditions.

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



The different images obtained by double labeling immunofluorescence microscopy of SAOS-2 (osteosarcoma) cell line shows that GRLF1 is not detected in this cellular tissue under these conditions.

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

Remaining cell lines tested gave a negative results under these conditons.