Product Specification Sheet

DDIT3/GADD 153 antibody

□ Cat # DDIT3H-A	Rabbit anti-Human GADD 153/DDIT3 antibody	**SIZE**: 100 µg
□ Cat # DDIT3M-A	Rabbit anti-Mouse GADD 153/DDIT3 antibody	**SIZE**: 100 µg

CHOP is a small nuclear protein that is capable of dimerizing with transcription factors C/EBP alpha and beta. GADD153 is a member of the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors. GADD153 can be found in the nucleus and its expression is induced by cellular stresses including nutrient deprivation, endoplasmic reticulum stress, and metabolic perturbations. The GADD153 protein functions as a dominant-negative inhibitor that inhibits DNA-binding activity with other C/EBP members, such as C/EBP and LAP (liver activator protein), by forming heterodimers. Fusion of GADD153 and FUS on chromosome 16 or EWSR1 on chromosome 22, induced by translocation, generates chimeric proteins in myxoid liposarcomas, also known as Ewing sarcoma. GADD153 is implicated in adipogenesis and promotes apoptosis in cells.

Under normal cellular conditions this protein is not expressed in detectable levels but is highly upregulated during times of cellular/ER stress. Examples of CHOP inducing stress include treatment with tunicamycin, nutrient starvation and reducing agents that interfere with the calcium flux across the ER membrane

Source of Antigen or Antibodies

Uniprot: DDIT3H-A: P35638 DDIT3M-A: P35639

Host: Rabbit

Clonality: Polyclonal

Purification: Ammonium sulfate followed by peptide affinity purification

Immunogen: Synthetic peptide derived from DDIT3 conjugated to KLH

Cross reactivity: The peptide (**DDIT3H-A)** used as an immunogen exhibits 100% homology with Rhesus, Gorilla, and Chimpanzee. 95% Orangutan. 91% Chinese Hamster, Goat, Sheep and Pig. 86% Rat. 82% Mouse.

The peptide (**DDIT3M-A)** used as an immunogen exhibits 95% homology with Rat and Bovine. 90% Human, Goat, and Sheep. 84% NHP, Cat, Horse, and Dog.

Subcellular Location: Nucleus and cytoplasm

Alternative names: DDIT-3, C/EBP zeta, C/EBP homologous protein, CCAAT/enhancer-binding protein homologous protein, Growth arrest and DNA damage-inducible protein GADD153

Recommended Secondary Antibody: Goat anti-Rabbit IgG-HRP (**ADI cat#20320)**

Form & Storage of Antibodies

□**Affinity pure IgG Solution**
Concentration: 0.5 mg/ml Volume: 200 µl
Supplied in PBS, pH 7.4 + 0.1% BSA
The antibody can be made available carrier free or conjugated to HRP, Biotin, or FITC on request

□ **Lyophilized powder**
Reconstitute powder in 200 µl distilled water to 0.5 mg/ml

Storage:

Short-term: 4°C for 1 month

Long-term: at –20°C or below in suitable aliquots after reconstitution for 1 year. Do not expose to multiple free/thaw cycles or store working, diluted solutions. Glycerol may be added to a final concentration of 50% and antibodies can be stored un-aliquoted at –20°C.

Recommended Usage

ELISA: Assay dependent concentration. Typically, between 0.1-2.0 µg/ml for capture/detection antibodies.

Western Blotting: 0.5-2.0 µg/ml
Predicted band size: 19.1 kDa
Observed band size: ~27 kDa.

IHC-P: 1-10 µg/ml. QC tested using 10 mM sodium citrate, pH 6 antigen retrieval. The antibody may work better with other retrieval solutions or no retrieval.

The above concentrations are a *suggestion*, user's must optimize their assay based on their own conditions. The antibody may work in other applications such as Immunocytochemistry or IP. These methods have not been tested by ADI.

This product is for In vitro research use only.

Related materials available from ADI

Catalog#	Description
BCL11-A	Rabbit Anti-Human BCL-2 antibody
BCL21-A	Rabbit Anti-Mouse BCL-2 antibody
BCL2-C	Recombinant BCL-2 control for Western blotting
AKT1-A	Rabbit anti-Human AKT1 antibody
AKT1-C	Recombinant MAPK1 control for Western blotting

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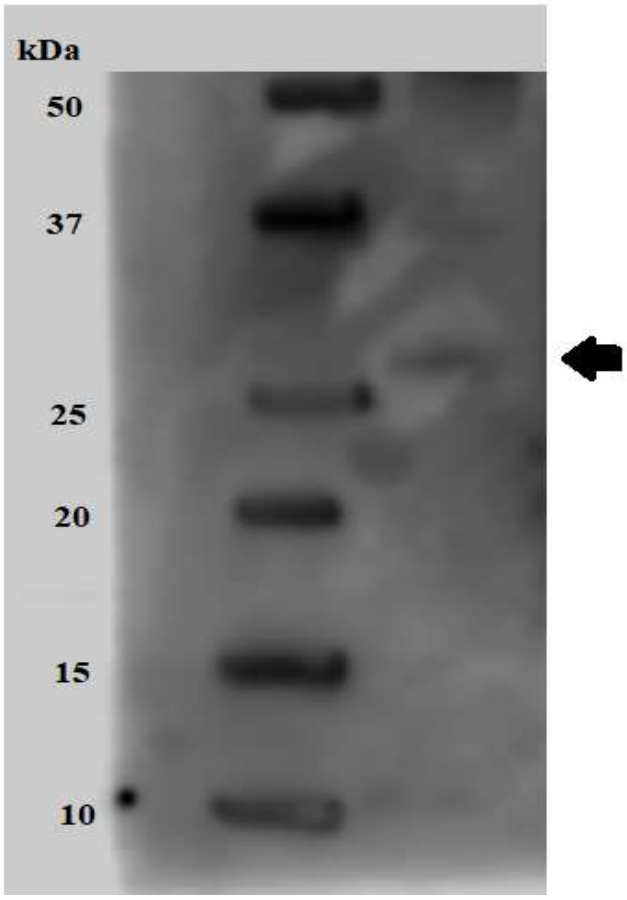
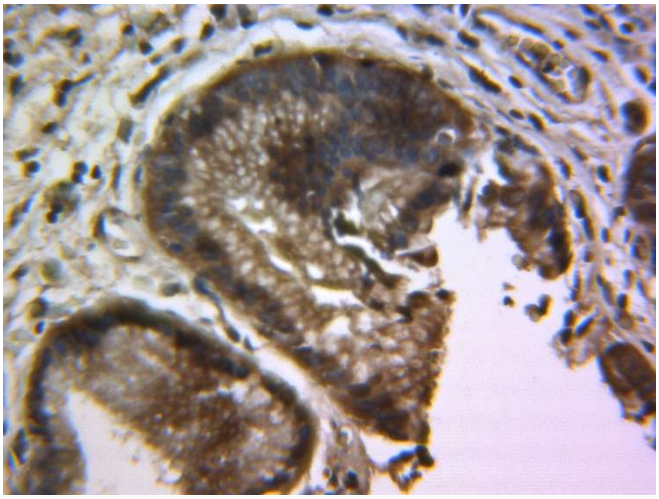
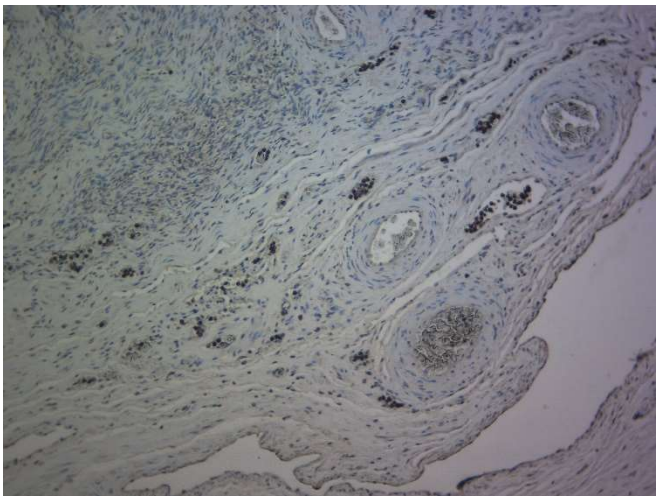
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com



Western blotting: 20 µg of a whole Human Ovary lysate was heated for 5 minutes at 95°C then electrophoretically separated on an 'Any kD' SDS-PAGE gel (Biorad). The gel was run at 100V for ~1 hour and 30 minutes then transferred to a 0.2 µm nitrocellulose membrane using the 'Low MW' settings on a Transblot Turbo (Biorad). The blot was blocked for 1 hour at room temperature with Fish plasma (Aquablock, EastCoastBio). **DDIT3H-A** was diluted with TBST+0.1% BSA to 1 ug/ml and incubated overnight at 4°C. The blot was washed with TBS-T 3 times for 5 minutes each. Goat anti-rabbit IgG HRP (**ADI cat#20320**) was diluted in TBST+0.1% BSA at a 1:10,000 dilution (50 ng/ml) then incubated for 1 hour at room temperature. The blot was washed 3 times with TBS-T for 5 minutes each. The blot was then incubated with ADI Femto ECL substrate (**ADI cat#80210**) for 5 minutes and imaged on a CCD imaging system (C-DiGit, LI-COR).





Immunohistochemistry: Human Gallbladder and Ovary slides were heated for 20 minutes at 60°C then deparrafinized. Antigen retrieval was performed for 10 minutes at 95°C in a microwave using 10 mM pH 6, sodium citrate buffer. The slide was then cooled for 20 minutes at room temperature before being blocked for 30 minutes with 2.5% normal goat serum. **DDIT3H-A** was diluted to 5 µg/ml in TBST+0.1% BSA and incubated overnight at 4°C. The slides were then washed twice and incubated with 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase. The slide was washed then incubated with Goat anti-Rabbit IgG HRP polymer detection reagent for 30 minutes at room temperature. The slide was washed twice, incubated with DAB for 3 minutes, washed with distilled water, then counterstained for 1 minute with Gil's II Hematoxylin before being cover-slipped.