DR4 Antibody
Cat. No.: 1139

Figure 1 Western Blot Validation in Human Cell Lines
Loading: 15 μg of lysates per lane. Antibodies: DR4 1139 (1 μg/mL), 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution. Lane A: H

Figure 2 Independent Antibody Validation (IAV) via Protein Expression Profile in Cell Lines
Loading: 15 μg of lysates per lane. Antibodies: DR4 1139 (1 μg/mL), DR4 1167 (4 μg/mL), and beta-actin (1 μg/mL), 1h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

Figure 3 Immunofluorescence Validation of DR4
Immunofluorescent analysis of 4% paraformaldehyde-fixed human spleen tissue labeling DR4 with 1139 at 20 μg/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red) and DAPI staining (blue). Image showing membrane staining on human spleen cells.

Figure 4 Immunocytochemistry Validation of DR4
Immunocytochemical analysis of 4% paraformaldehyde-fixed Jurkat cells labeling DR4 with 1139 at 10 μg/mL, followed by goat anti-rabbit IgG secondary antibody at 1/250 dilution (red). Image showing membrane staining on Jurkat cells.

Figure 5 Immunohistochemistry Validation of DR4
Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-DR4 antibody (1139) at 10 μg/mL. Tissue was fixed with formaldehyde and blocked with 10% serum for 1 h at RT, antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4μ C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.
The expression of DR4 was knocked down via DR4 siRNA, 24 h later cells were treated with dipyridamole for 24 h. DR4 protein expression detected by anti-DR4 antibodies (1139) was disrupted. Dipyridamole up-regulated the expression of DR4.

DR4 protein expression detected by anti-DR4 antibodies (1139) was increased after transient brain ischemia (tMCAO) and decreased after pre-conditioning stimulus. Confocal microscopic images displaying NeuN (a,d,g) (green), DR4 (b,e,h) (red), and Merge (c,f,i) (yellow) in the brain peri-ischemic region of rats after 5 h.

MeWo melanoma cells were exposed to affinity-purified MDA7/IL-24. After 48 h of treatment, cells were collected and cytospins prepared for cytochemical assessment of their TRAIL receptor (R1 and R2) expression (anti-DR4 (1139) or anti-DR5, AEC, hematoxylin). Both DR4 and DR5 expression were upregulated in MeWo cells after treatment.

HeLa cells were transfected with DR4 siRNA or LacZ control siRNA. At 24 h after transfection, the cells were treated with or without 20 μM luteolin for 24 h. Western blot analysis was carried out with anti-DR4 antibodies (1139). DR4 expression was markedly reduced after DR4 knockdown.

**Specifications**

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<thead>
<tr>
<th>HOST SPECIES:</th>
<th>Rabbit</th>
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<tr>
<td>SPECIES REACTIVITY:</td>
<td>Human, Mouse, Rat</td>
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<td>IMMUNOGEN:</td>
<td>Anti-DR4 antibody (1139) was raised against a peptide corresponding to 19 amino acids near the carboxy terminus of human DR4. The immunogen is located within the last 50 amino acids of DR4.</td>
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TESTED APPLICATIONS: ELISA, ICC, IF, IHC-P, WB

APPLICATIONS: WB: 1 μg/mL; IHC-P: 5 μg/mL; IHC: 10 μg/mL; IF: 20 μg/mL.

Antibody validated: Western Blot in human and mouse samples; Immunohistochemistry in human samples; Immunocytochemistry in human samples and Immunofluorescence in human and rat samples. All other applications and species not yet tested.

SPECIFICITY: DR4 antibody has no cross reaction to DR5

POSITIVE CONTROL: 1) Cat. No. 1202 - A431 Cell Lysate
2) Cat. No. 1203 - A549 Cell Lysate
3) Cat. No. 1224 - Daudi Cell Lysate
4) Cat. No. 10-901 - Human Spleen Tissue Slide

PREDICTED MOLECULAR WEIGHT: Predicted: 50kD
Observed: 55kD (Post-modification: 1 N-linked glycosylation)

Advanced Validation

Independent Antibody Validation in Cell lines (Figure 2) shows similar DR4 expression profile in human cell lines detected by two independent anti-DR4 antibodies that recognize different epitopes, 1139 against C-terminus domain and 1167 against the N-terminus domain. DR4 proteins are detected in all the tested cell lines except CaCo-2 at different expression levels by the two independent antibodies.

KD validation (Figure 6, 9, 10): Anti-DR4 antibody (1139) specificity was further verified by DR4 specific siRNA knockdown. DR4 signal in SW480, Huh7 and HeLa cells transfected with DR4 siRNAs was disrupted in comparison with that in cells transfected with control siRNAs.

ISOFORMS: Human DR4 has only 1 isoform (468aa, 50kD).

Properties

PURIFICATION: DR4 Antibody is Antibody is affinity chromatography purified via peptide column.

CLONALITY: Polyclonal

ISOTYPE: IgG

CONJUGATE: Unconjugated

PHYSICAL STATE: Liquid

BUFFER: DR4 Antibody is supplied in PBS containing 0.02% sodium azide.

CONCENTRATION: 1 mg/mL

STORAGE CONDITIONS: DR4 antibody can be stored at 4μC for three months and -20μC, stable for up to one year. As with all antibodies care should be taken to avoid repeated freeze thaw cycles. Antibodies should not be exposed to prolonged high temperatures.
**Official Symbol:** TNFRSF10A

**Alternate Names:** DR4 Antibody: DR4, APO2, CD261, TRAILR1, TRAILR-1, DR4, Tumor necrosis factor receptor superfamily member 10A, Death receptor 4, TRAIL receptor 1

**Accession No.:** AAC51226

**Protein GI No.:** 1945072

**Gene ID:** 8797

**User Note:** Optimal dilutions for each application to be determined by the researcher.

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**Background and References**

**Background:**

DR4 Antibody: Apoptosis, or programmed cell death, occurs during normal cellular differentiation and development of multicellular organisms. Apoptosis is induced by certain cytokines including TNF and Fas ligand in the TNF family through their death domain containing receptors, TNFR1 and Fas. A novel death domain containing receptor was recently identified and designated DR4 (for death receptor 4). The ligand for this novel death receptor has been identified and termed TRAIL2, 3, which is a new member in the TNF family. DR4 is also called TRAIL receptor-1 (TRAIL-R1). DR4 is expressed in most of human tissues including spleen, peripheral blood leukocytes, small intestine and thymus. Like TNFR1, Fas and DR3, DR4 mediates apoptosis and NF-κB activation in many tissues and cells.

**References:**

1) Pan et al. Science 1997;276:111-3


4) Schneider et al. Immunity 1997; 7:831-6

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**Citations**


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<td>16)</td>
<td>Taniguchi et al. Targeting the glyoxalase pathway enhances TRAIL efficacy in cancer cells by downregulating the expression of antiapoptotic molecules. Mol Cancer Ther. 2012;11(10):2294-300. PMID: 22784708</td>
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<tr>
<td>19)</td>
<td>Rizzardi et al. Apoptosis-related factors (TRAIL, DR4, DR5, DcR1, DcR2, apoptotic cells) and proliferative activity in ameloblastomas. Anticancer Res.2009;29(4):1137-42. PMID: 19414356</td>
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