ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Anti-Mouse Transferrin (Tf) Antibodies

□ Cat. # TF14-A	**Goat** Anti-Mouse Transferrin IgG	**SIZE**: 100 ug
□ Cat. # TF14-HRP	**Goat** Anti-Mouse Transferrin IgG-HRP Conjugate	**SIZE**: 100 ug
□ Cat. # TF14-BTN	**Goat** Anti-Mouse Transferrin IgG-Biotin Conjugate	**SIZE**: 50 ug
□ Cat. # TF14-C	Purified **Mouse** Transferrin protein W. Blot +ve control	**SIZE**: 100 ul

Elemental iron is required for a variety of normal cellular functions and vital for proper growth and development. However, natural iron is quite insoluble and excess iron is harmful, since it can catalyze the formation of potentially damaging reactive oxygen species. The major pool of body iron (~85%; 40-50 mg/kg) is found in circulating hemoglobin and muscle myoglobin. Iron absorption occurs primarily in the intestine (duodenum) and inversely related to body iron reserve. Several proteins including **Ferritin, transferrin (Tf), transferrin receptors (TfRs)**, and **iron regulatory proteins (IRPs**) etc play a key role in iron metabolism.

Transferrin (Tf, human chromosome 3, 679 aa), a serum glycoprotein of ~80 kDa and synthesized in the liver, is the primary protein of inter-organ transport of nonheme iron. Tf can bind two iron atoms. Tf binds to membrane **Transferrin receptors (TfRs)** and taken up by endocytosis. Iron is released from Tf, within acidic endosomes, into the cytoplasm apparently through the action of DMT1. The apoTf-TfR complex is returned to the cell surface, where, apo-Tf dissociates from TfR at the extracellular pH. The classical TfR, now termed **TfR1**, is a homodimeric (95 kDa subunits) type II membrane glycoprotein that binds two molecules of Tf. Human TfR1 (human 760 aa; mouse 763 aa) has a cytoplasmic domain 1-67aa, 68-88 aa TM, and 89-760 aa as extracellular domains. A monomeric serum form or **soluble TfR1** (~80 kDa) also exists that lacks residues 1-100 aa. Recently, a second Tf receptor, **TfR2**, has been cloned and characterized. TfR2 shares 45% identity with TfR1, and 27% with PMSA. Several variants of Tf have been identified with varying iron binding ability.

Source of Antigen and Antibodies

Purified Mouse serum Tf (Cat # TF14-N-1) was used to immunized goat Antibodies have been purified over the antigen column (**Cat # TF14-A**). Purified IgG is supplied PBS pH 7.4, 0.05% azide in 100 ug/vial (1 mg/ml) in solution or powder.

Cat# TF14-HRP, HRP-conjugate

Purified antibody was coupled to HRP (RZ>3.0) using periodate method. The molar enzyme to protein (E/P) ratio = 4.0. The antibody is supplied in stabilizing buffer, 0.1% BSA, 0.1% prolcin-300 as preservative in either **lyophilized** (0.1 ml) or **liquid** form (0.1 ml, 0.2-0.5 mg/ml). Reconstitute powder in PBS in 0.1 ml. Store at 4oC in suitable aliquots. Stability is ~6-12 months. Do not freeze and thaw.

Suggested conjugate dilutions are 1:1,000-1:10,000 ELISA, 1:1K-1:5K for western, and 1:200-1:1000 (IHC).

Cat# TF14-BTN, Biotin-conjugate

Purified anti-Mouse Tf antibody was coupled to Biotin using Biotinamidocaproate N-Hydroxysuccinimide Ester (BAC**)** at F/P ratio ~10-20:1. The antibody is supplied in PBS, pH 7.4, 0.2% BSA and 0.05% azide in either **lyophilized** (50 ug) or **liquid** form (50 ug/50 ul). Reconstitute powder in PBS in 0.1 ml to prepare 0.5 mg/ml solution. Store at -20oC in suitable aliquots. Stability is ~6-12 months. Do not freeze and thaw.
Suggested conjugate dilutions are 1:5,000-1:30,000 ELISA, 1:2K-1:10K for western.

Highly purified mouse serum apo-Transferrin (Tf, mol wt 76-80 kda was used as positive control. For Western blot +ve control (**Cat # TF14-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **TF14**-C for good visibility with antibody Cat # **TF14**-S or #TF14-A. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **TF14**-C solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder.

Recommended Usage

Western Blotting (1:1K-5K antibody using ECL technique). Tf is ~80 kDa.

ELISA: coat ELISA plates at 1 ug/ml and detected with antibodies (1:1K-10K for neat serum and 1-5 ug/ml for IgG).

Histochemistry & Immunofluorescence: Not tested.

Specificity & Cross-reactivity

Anti-Mouse Tf antibodies reacts with Mouse and poorly with human and rat. We recommend using other antibodies for human (cat# TF11-A) and rat (TF12-S). Antibody cross-reactivity in various other species has not been studied. Purified human, mouse, and rat Tfs proteins are available for use as positive controls.

General References: Bowman, B. H. et al (1988) Adv. Genet. 25: 1-38; Evans, R. W. et al (1982) Biochem. J. 201: 19-26; MacGillivray, R. T. A et al (1982) PNAS 79: 2504-2508; Park, I. et al (1985) PNAS 82, 3149; Uzan, G. et al (1984) BBRC 119, 273; Yang, F. et al (1984) PNAS 81, 2752-2756; Nelson N et al (1999) EMBO J. 18, 4361(review); Cairo G et al (2000) Biochem. J. 352, 241-250

*This product is for In vitro research use only.

Related material available from ADI

Mouse albumin, IgG, CRP, IgG, IgA, IgM, and IgE ELISA kits

TF14-S, -A, -C, -HRP, -BTN 70501A

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