

HyTest NEWS



Transferrin and Transferrin receptor



Soluble transferrin receptor, transferrin and their complex

Cells obtain iron from plasma where it circulates in a complex with a carrier protein transferrin (Tf). To be transported into cells, iron loaded Tf is bound to transferrin receptor (TfR), and their complex passes into cells by means of internalization, where iron releases by pH-dependent mechanism (1).

Transferrin receptor is a transmembrane protein that participates in iron transport from plasma into cells. It consists of two identical subunits of 95 kDa linked by two disulfide bonds. Each TfR subunit contains an N-terminal cytoplasmic domain (1-67 amino acid residues), a transmembrane domain (68-88 amino acid residues) and a C-terminal extracellular domain (89-760 amino acid residues) (2).

The main pool of TfR molecules is located on erythroblasts which demand a lot of iron for hemoglobin synthesis. After the erythroid cells have matured, the extracellular part of the TfR molecule is truncated from the cell surface by cleavage of an R100 – L101 bond. TfR released into the blood stream consists of 101-760 amino acid residues of cell TfR and is called soluble (or serum) transferrin receptor (sTfR) (3).

The expression of transferrin receptor depends on the concentration of iron in the cellular cytoplasm. The concentration of soluble transferrin receptor

(sTfR) has been reported to be proportional to the total amount of cell-associated transferrin receptor.

In blood, soluble TfR is completely bound to Tf and circulates as sTfR-Tf complex.

The determination of the sTfR level in blood has become widely used in clinical practice (4 – 7). The normal concentration of sTfR in blood ranges within 2 – 5 $\mu\text{g/ml}$. An increase in the sTfR level was found in iron deficiency anemia, autoimmune hemolytic anemia, hereditary spherocytosis, β -thalassemia, sickle cell anemia and some others. Soluble TfR is indispensable marker of iron deficiency anemia and is mainly used for the differentiation between iron deficiency anemia (accompanied by an increase in the sTfR level) and anemia of chronic disease (proceeded at the normal sTfR level) (8).

The measurement of Tf is also widely used in diagnosis of anemia together with the determination of sTfR, ferritin and iron concentration in serum.

Soluble transferrin receptor and transferrin are measured in plasma and serum by immunoassays based on the specific anti-Tf or anti-TfR antibodies.

HyTest offers anti-TfR and anti-Tf MAbs, allowing detection of TfR, Tf and sTfR-Tf complex in human blood.



Anti-transferrin receptor monoclonal antibodies

Host animal:	Mice Balb/c
Cell line used for fusion:	Sp2/0
Antigen:	Human TfR
Purification method:	Protein A affinity chromatography
Presentation:	MAB solution in PBS with 0.1 % sodium azide
Application:	TfR immunoassay, TfR immunoaffinity purification and TfR immunodetection in Western blotting

Hybridoma cell lines producing MAbs were derived from hybridization of Sp2/0 myeloma cells with spleen cells of Balb/c mice immunized with purified human placental TfR. Specificity of antibodies was confirmed by ELISA and Western blotting. MAbs 2B6, 5B11, 7F8, 11F5, 10D2, 13E4 and 23D10 recognize placental TfR (pTfR) and soluble TfR in

ELISA. All these MAbs recognize sTfR in Western blotting after SDS gel electrophoresis under non-reducing conditions. MAb 7-1 recognizes pTfR in ELISA. MAb 9H4 recognizes pTfR in Western blotting after SDS gel electrophoresis under both reducing and non-reducing conditions.

See ordering information on page 4.

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See ordering information on page 4.



Applications

1. Transferrin (Tf) sandwich immunoassay

Recommended pairs to be used for Tf detection in human plasma (serum) by sandwich immunoassay (capture – detection):

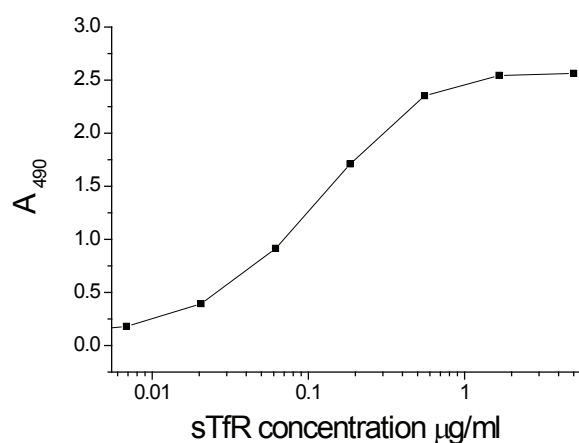
1C10 – 2A2
 1C10 – 12A6
 8B9 – 11D3
 8B9 – 12A6
 11D3 – 1C10
 11D3 – 8B9

2. Soluble transferrin receptor (sTfR) sandwich immunoassay

Recommended pairs to be used for sTfR detection in human plasma (serum) by sandwich immunoassay (capture – detection):

23D10 – 13E4
 11F5 – 7F8
 2B6 – 11F5
 7F8 – 23D10
 10D2 – 13E4
 5B11 – 2B6
 10D2 – 13E4

20 ng/ml assay sensitivity could be reached.



3. Detection of sTfR-Tf complex in sandwich immunoassay

The determination of sTfR-Tf complex in plasma or serum is based on using anti-sTfR MAbs for capture and anti-Tf MAbs for detection.

Recommended pairs to be used for sTfR-Tf complex detection in human plasma (serum) by sandwich immunoassay (capture – detection):

23D10 (a-TfR Cat.# 4Tr26) – 8B9 (a-Tf Cat.# 4T15)
 23D10 (a-TfR Cat.# 4Tr26) – 11D3 (a-Tf Cat.# 4T15)

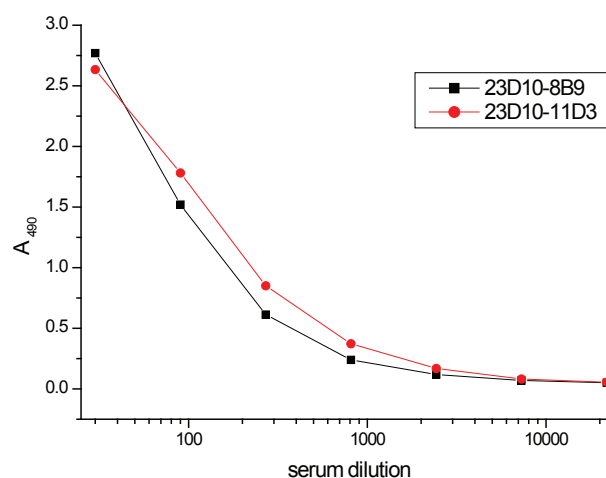


Figure 2. Titration curve of sTfR-Tf complex contained in normal human serum using sTfR-Tf assay.

Capture: MAb 23D10; 200 ng/well
 Detection: MAb 8B9 or 11D3 conjugated with HRP
 Room temperature

Figure 1. Titration curve of purified soluble transferrin receptor (sTfR) using pair 23D10 – 13E4.

Capture: MAb 23D10; 200 ng/well
 Detection: MAb 13E4 conjugated with HRP
 Room temperature

Ordering information:

Product	Cat.#	MAb	Subclass	Application
Anti-transferrin receptor	4Tr26	2B6	IgG2a	Sandwich immunoassay, WB
Anti-transferrin receptor	4Tr26	5B11	IgG2b	Sandwich immunoassay, WB
Anti-transferrin receptor	4Tr26	7F8	IgG2a	Sandwich immunoassay, WB
Anti-transferrin receptor	4Tr26	11F5	IgG2b	Sandwich immunoassay, WB
Anti-transferrin receptor	4Tr26	10D2	IgG2b	Sandwich immunoassay, WB
Anti-transferrin receptor	4Tr26	13E4	IgG2a	Sandwich immunoassay, WB
Anti-transferrin receptor	4Tr26	23D10	IgG2b	Sandwich immunoassay, WB
Anti-transferrin receptor	4Tr26	7-1	IgG2b	Sandwich immunoassay

Ordering information:

Product	Cat.#	MAb	Subclass	Application
Anti-transferrin	4T15	1C10	IgG1	Sandwich immunoassay
Anti-transferrin	4T15	2A2	IgG1	Sandwich immunoassay
Anti-transferrin	4T15	8B9	IgG1	Sandwich immunoassay
Anti-transferrin	4T15	11D3	IgG1	Sandwich immunoassay
Anti-transferrin	4T15	12A6	IgG1	Sandwich immunoassay

References:

1. Feelders RA, Kuiper-Kramer EPA, Eijk HG van. Structure, function and clinical significance of transferrin receptors. Clin Chem Lab Med 1999; 37(1):1-10.
2. Schneider C, Owen MJ, Banville D, Williams JG. Primary structure of human transferrin receptor deduced from the mRNA sequence. Nature 1984; 311(5987):675-8.
3. Shih YJ, Baynes RD, Hudson BG, Flowers CH, Skikne BS, Cook JD. Serum transferrin receptor is a truncated form of tissue receptor. J Biol Chem 1990; 265(31):19077-81.
4. Flowers CH, Skikne BS, Covell AM, Cook JD. The clinical measurement of serum transferrin receptor. J Lab Clin Med 1989; 114(4):368-77.
5. Punnonen K, Irjala K, Rajamaki A. Iron-deficiency anemia is associated with high concentrations of transferrin receptor in serum. Clin Chem 1994; 40(5):774-6.
6. Cook JD. The measurement of serum transferrin receptor. Am J Med Sci 1999; 318(4):269-76.
7. Koulaouzidis A, Said E, Cottier R, Saeed AA. Soluble transferrin receptors and iron deficiency, a step beyond ferritin. A systematic review. J Gastrointest Liver Dis. 2009;18(3):345-52.
8. Ferguson BJ, Skikne BS, Simpson KM, Baynes RD, Cook JD. Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. J.Lab.Clin. Med 1992; 19:385-90.



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