

Transferrin, Transferrin Receptor 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 hemoglo-bin 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 mg/ml. An increase in the sTfR level was found in iron deficiency anemia, autoimmune hemolytic anemia, hereditary spherocytosis, b-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.

We offer anti-TfR and anti-Tf MAbs, allowing detection of TfR, Tf and sTfR-Tf complex in human blood. We also offer soluble transferrin receptor antigens.

MONOCLONAL ANTIBODIES SPECIFIC TO TRANSFERRIN RECEPTOR

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, 11F5cc, 13E4cc 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.

MONOCLONAL ANTIBODIES SPECIFIC TO TRANSFERRIN

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 Tf. Specificity of antibodies was confirmed by ELISA. All antibodies recognize Tf in ELISA.

SOLUBLE TRANSFERRIN RECEPTOR ANTIGEN

We provide a recombinant antigen expressed in mammalian cell line.

Transferrin (Tf) sandwich immunoassay

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

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

Soluble transferrin receptor (sTfR) sandwich immunoassay

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

23D10 – 13E4cc	2B6 – 11F5cc
----------------	--------------

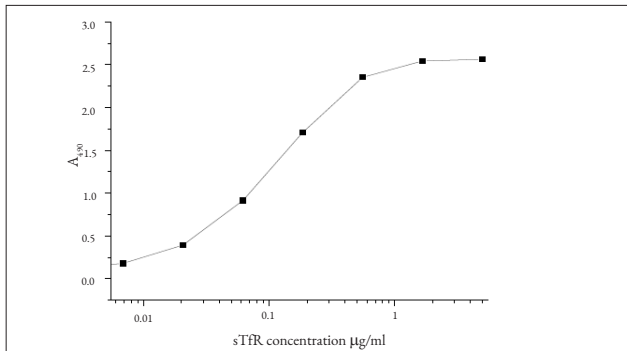


Figure 1. Titration curve of purified soluble transferrin receptor (sTfR) using pair 23D10 – 13E4. Capture MAb 23D10; 200 ng/well, Detection MAb 13E4 conjugated with HRP, Room temperature

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 – 8B9	23D10 – 11D3
-------------	--------------

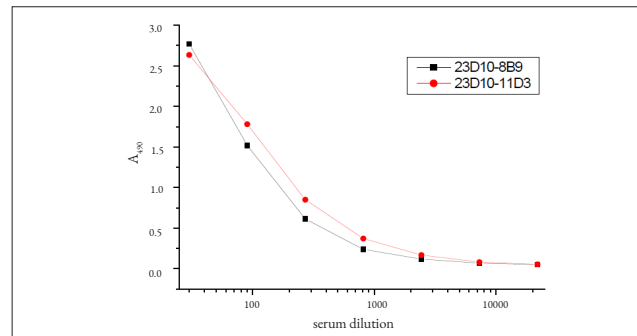


Figure 2. Titration curve of sTfR-Tf complex contained in normal human serum using sTfR-Tf assay. Capture MAb 23D10; 200 ng/well, Detection MAb 8B9 or 11D3 conjugated with HRP, Room temperature.

REFERENCES

1. **Feelders RA et al.** Structure, function and clinical significance of transferrin receptors. *Clin Chem Lab Med* 1999; 37(1):1-10.
2. **Schneider C et al.** Primary structure of human transferrin receptor deduced from the mRNA sequence. *Nature* 1984; 311(5987):675-8.
3. **Shih YJ et al.** Serum transferrin receptor is a truncated form of tissue receptor. *J Biol Chem* 1990; 265(31):19077-81.
4. **Flowers CH et al.** The clinical measurement of serum transferrin receptor. *J Lab Clin Med* 1989; 114(4):368-77.
5. **Punnonen K et al.** 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 et al.** 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 et al.** Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. *J.Lab.Clin.Med* 1992; 19:385-90.

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Transferrin	4T15	1C10	IgG2b	EIA
		8B9	IgG2b	EIA, WB
		11D3	IgG2b	EIA, WB
		12A6	IgG2b	EIA, WB
Transferrin receptor	4Tr26	2B6	IgG2a	EIA, WB
		23D10	IgG2b	EIA, WB
Transferrin receptor	4Tr26cc	11F5cc	IgG2b	<i>In vitro</i> , EIA, WB
		13E4cc	IgG2a	<i>In vitro</i> , EIA, WB

ANTIGEN

Product name	Cat. #	Purity	Source
Transferrin receptor, soluble, recombinant	8ST6	>95%	Recombinant

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced *in vivo*. MAbs produced *in vitro* are expected to have similar performance.