

## Technical Data Sheet

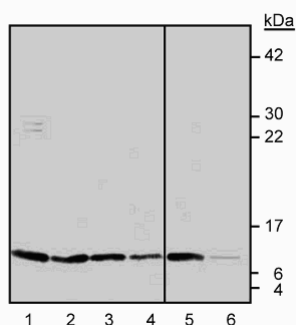
## Purified Mouse Anti-Human Thioredoxin

## Product Information

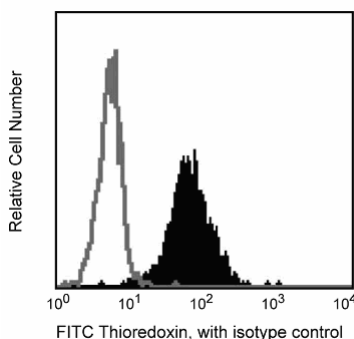
<b>Material Number:</b>	559969
<b>Alternate Name:</b>	TRX (1-104)
<b>Size:</b>	0.1 mg
<b>Concentration:</b>	0.5 mg/ml
<b>Clone:</b>	2G11/TRX
<b>Immunogen:</b>	Recombinant human Trx
<b>Isotype:</b>	Mouse IgG1
<b>Reactivity:</b>	QC Testing: Human
<b>Storage Buffer:</b>	Aqueous buffered solution containing ≤0.09% sodium azide.

## Description

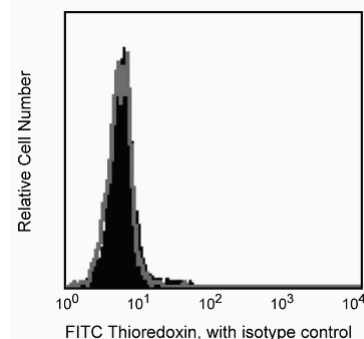
The immunogen used to raise the 2G11 hybridoma was recombinant full length human thioredoxin (Trx) is an intracellular, multifunctional 12 kD (104 a.a.) protein with reduction/oxidation (redox) activity expressed by most cells of the human body. Trx is also secreted via the leaderless pathway (i.e. like IL-1 $\beta$ ) by cells such as monocytes and lymphocytes upon activation or in response to oxidative stress. A truncated, 10 kD form of Trx (Trx1-84) has also been reported. Secreted Trx (also known as adult T-cell leukemia derived factor (ADF)) exhibits several cytokine- and chemokine-like functions such as activating eosinophils and granulocytes and chemotaxis for neutrophils, monocytes and T cells. 2G11 reacts exclusively with Trx(1-104), but not with its truncated form.



**Detection of Trx (1-104) by Western Blotting:** Recombinant huTrx (100, 50 25 or 12.5 ng-lanes 1 -4, respectively) or dilutions of U937 cell lysate ( $5 \times 10^4$  or  $2.5 \times 10^4$  cell equivalent-- lanes 5 and 6 respectively) were separated on 14% SDS- PAGE under reducing conditions. The separated proteins were transferred to PVDF membrane, and probed with 2G11 (1  $\mu$ g/ml). AKP-conjugated polyclonal anti- mouse IgG was used as a secondary antibody at 1:1000 dilution, and the blot was developed with BCIP/NBT substrate (Sigma). The 2G11 antibody detects a single, approx. 12 kDa band protein in lanes 1 - 4 (huTrx) as well as in the cellular lysate, that corresponds to the MW of Trx. (left panel).



**Expression of Trx by human peripheral blood mononuclear cells (PBMC).** Human PBMC were fixed, permeabilized, and subsequently stained with 0.125  $\mu$ g of FITC-conjugated mouse anti-human Trx antibody (FITC-2G11). A histogram overlay shows specific cell staining compared to IgG1 isotype control FITC-MOPC-21 (Cat. No. 554679) (middle panel). To demonstrate specificity of staining, the staining by FITC-2G11 was blocked by preincubation with an excess of the unlabeled 2G11 antibody (10  $\mu$ g, Cat. No. 559969) prior to staining with the FITC-2G11 antibody (right panel).



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## Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Store undiluted at 4° C.

## Application Notes

### Application

Western blot	Routinely Tested
Intracellular staining (flow cytometry)	Routinely Tested

### Recommended Assay Procedure:

The 2G11 antibody is suitable to detect Trx (1-104) in western blots. 2G11 is useful for Intracellular Staining and Flow cytometry using appropriate conjugated secondary antibody. A useful control for demonstrating specificity of staining with conjugated 2G11 is to pre-block the fixed/permeabilized cells with unlabeled 2G11 antibody (Cat. No. 559969) prior to staining, see figure.

## Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
554121	Purified Mouse IgG1 $\kappa$ Isotype Control	0.1 mg	MOPC-21
555988	FITC Goat Anti-Mouse IgG/IgM	0.5 mg	Polyclonal
554714	BD Cytotfix/Cytoperm Fixation/Permeablization Kit	250 tests	(none)

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

## References

- Bertini R, Howard OM, Dong HF, et al. Thioredoxin, a redox enzyme released in infection and inflammation, is a unique chemoattractant for neutrophils, monocytes, and T cells. *J Exp Med.* 1999; 189(11):1783-1789.(Biology)
- Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128.(Methodology: IC/FCM Block)
- Sahaf B, Söderberg A, Spyrou G, et al. Thioredoxin expression and localization in human cell lines: detection of full-length and truncated species. *Exp Cell Res.* 1997; 236(1):181-192.(Biology)
- Yoshida S, Katoh T, Tetsuka T, Uno K, Matsui N, Okamoto T. Involvement of thioredoxin in rheumatoid arthritis: its costimulatory roles in the TNF-alpha-induced production of IL-6 and IL-8 from cultured synovial fibroblasts. *J Immunol.* 1999; 163(1):351-358.(Biology)