

Product Specification Sheet

Thioredoxin (fusion protein) Antibodies

Cat # THR11-AS

Rabbit Anti-Thioredoxin (E. Coli) IgG-Agarose
FORM: Soln

SIZE: 0.25 ml
Lyophilized

Recombinant DNA technology allows the addition of short pieces of well-defined tags, "peptides" or proteins at the amino or c-terminus of target genes, which can provide 'affinity handles' designed to bind specific matrices. Therefore, tags enable a selective identification and purification of the protein of interest. Eukaryotic genes are often cloned into E. coli thioredoxin gene, resulting in the expression of a desired protein as a fusion hybrid with thioredoxin (1). Thioredoxin is a small electron transport protein that serves as hydrogen donor in the enzymatic reduction of ribonucleotides to deoxyribonucleotides (1). Thioredoxin from E. coli consists of a single polypeptide of 108 aa (~11.7 kDa). The gene encoding Thioredoxin has been clones into several plasmids and serves as a fusion partner for the expression of other clones genes. The system often produces high expression of soluble fusion proteins in the E. coli cytoplasm. The fusion proteins may also fold correctly with biological activity.

Anti-Thioredoxin antibodies allow a simple isolation of fusion proteins directly from the crude bacterial lysate, using immunoaffinity chromatography (1) or used in immunoprecipitation. Anti- thioredoxin can also be used for the immunocytochemical detection of thioredoxin in cells and tissues that express transfected bacterial thioredoxin gene (1). Anti-thioredoxin may be used in various immunoassays to identify the expression of thioredoxin fusion protein.

Source of Antigen and Antibodies

Rabbits were injected with **recombinant purified E. coli thioredoxin (Cat # THR15-R)** to obtain polyclonal antiserum (**cat # THR11-S**). The antiserum has been affinity purified (**cat # THR11-A**) using the purified THR-coupled to Agarose affinity column.

Purified anti-THR IgG was coupled to Sepharose at 2-3 mg/ml bed volume (**Cat # THR11-AS**). It is supplied in PBS+0.05% azide as 1:1 (v/v) suspension. Store at 2-4oC. **Do not freeze.**

Recommended Usage

Anti-tag IgG-Agarose may be used for immunoprecipitation and for affinity purification of fusion proteins containing the tag. The binding capacity of the column for the affinity purification of the fusion protein must also be evaluated for a given fusion protein.

Immunoprecipitation

25-50 ul of gel volume (50-10 ul of 1:1 suspension) per 500 ug of the protein lysate. For recombinant proteins with high level of expression, the lysate amounts must be optimized.

Purification of THR1-fusion proteins.

The purification can be performed using a small column or the batch process. Purification can be performed at 4oC (for temp sensitive proteins) or room temp.

1. Apply clear cell lysates to the column and recycle 2-3 times. Cell extracts can be prepared in PBS, pH 7.4 or other suitable buffers.
2. Collect the flow through, and wash until the OD280 is <0.020 or achieved a base line.
3. Elute proteins with 0.1M ammonium hydroxide (pH 11-12) into vials containing 30-50 ul of 1N acetic acid per ml of eluant. Collect 0.5-1 ml fraction (5-10 ml total).
4. Low pH elution (Tris-Gly pH 2.5, followed by neutralization in 1M Tris pH 8.0) can also be used.
5. Affinity column should not be exposed to low or high pH for prolonged periods of time.
6. Binding and elution buffers must be optimized for a given protein.
7. Bound proteins should be dialyzed against PBS or other buffer at 4oC and concentrated if necessary.

Antibody concentration must be optimized for each application under defined experimental conditions.

Stability: 6-12 months at -20oC or below.

Shipping: 4oC for solutions and room temp for powder.

General References: Narayanan, S., J. Chromatogr., 658, 237 (1994), Casey, J., et al., J. Immunol. Meth., 179, 105 (1995), Uhlen, M., and Moks, T., Meth. Enzymol., 185, 129 (1990), Skerra, A., et al., Bio/Technology, 9, 273 (1991), Holmgren A (1985) Ann Rev. Biochem. 54, 237; Rosen A et al (1995) Int. Immunol. 7, 625

*This product is for In vitro research use only.

Other Fusion tag antibodies available from ADI

Anti-MBP, Poly-His, GST, beta-Gal, VSV-G, Flag, HA-tag, and c-myc

THR11-AS 60615A