## DESCRIPTION

**Source**  
*E. coli*-derived  
Val2-Val105  
Accession # P10599

**N-terminal Sequence Analysis**  
Val2

**Predicted Molecular Mass**  
12 kDa

## SPECIFICATIONS

**SDS-PAGE**  
12 kDa, reducing conditions

**Activity**  
Measured by its ability to catalyze the reduction of insulin. The reaction leads to precipitation, which can be measured by absorbance at 650 nm. The specific activity is >10 A<sub>650</sub>/min/mg, as measured under the described conditions.

**Endotoxin Level**  
<0.10 EU per 1 μg of the protein by the LAL method.

**Purity**  
>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

**Formulation**  
Lyophilized from a 0.2 μm filtered solution in PBS, EDTA and DTT. See Certificate of Analysis for details.

## Activity Assay Protocol

### Materials
- **Assay Buffer:** 50 mM MES, 250 mM NaCl, 2 mM EDTA, pH 6.5
- **Recombinant Human Thioredoxin-1 (hTRX-1) (Catalog # 1970-TX)**
- **Human Insulin (Sigma, Catalog # I-9278)**
- **Dithiothreitol (DTT) (Sigma, Catalog # D-0632), 1 M stock in deionized water**
- **96-well Clear Plate (Costar, Catalog # 92592)**
- **Plate Reader (Model: Spectramax Plus by Molecular Devices) or equivalent**

### Assay
1. Dilute rhTRX-1 to 20 μM in Assay Buffer.
2. Dilute hInsulin to 520 μM in Assay Buffer.
3. Dilute DTT to 55 mM in Assay Buffer.
4. Load into a clear plate 50 μL of Assay Buffer, 25 μL of 20 μM hTRX-1, and 25 μL of 520 μM hInsulin. For a Substrate Blank, load 75 μL of Assay Buffer and 25 μL of 520 μM hInsulin. Start the reaction by adding 10 μL of 55 mM DTT to all wells.
5. Read at A<sub>650</sub> in kinetic mode for 15 minutes. Use the linear portion of the reaction to determine specific activity.
6. Calculate specific activity:

   \[
   \text{Specific Activity (Abs/min/mg)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (Abs/min)}**}{\text{amount of enzyme (mg)}}
   \]

   *Adjusted for Substrate Blank  
   **Note: the output of many spectrophotometers in kinetic mode is in mOD

### Final Assay Conditions
- **Per Well:**  
  - rhTRX-1: 0.00585 mg  
  - hInsulin: 118 μM  
  - DTT: 5 mM

## PREPARATION AND STORAGE

**Reconstitution**  
Reconstitute at 1000 μg/mL in sterile PBS.

**Shipping**  
The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage**  
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.  
- 6 months from date of receipt, -20 to -70 °C as supplied.  
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.
Thioredoxins (Trxs) are a group of small ubiquitous proteins in all living cells that are key regulators of cellular redox balance (1, 2). The mammalian Trx family has three members. The Trx-1, which is a secreted and cellular protein, the mitochondria-specific Trx-2, and the Trx-like cytosolic protein p32TrxL (3-5). The active site of mammalian Trxs contains two cysteines in the conserved sequence -Y-C-G-P-C-K-. In Trx-1 the conserved cysteine residues are in positions 32 and 35, respectively. Trxs exist either in a reduced or in an oxidized state when the two cysteines at the active site form an intramolecular disulfide bridge. NADPH and the flavoprotein thioredoxin reductase can convert the oxidized Trx into the reduced Trx. Trx-1 is the only extracellular occurring thioredoxin, and is secreted by lymphocytes, hepatocytes, fibroblasts, and several tumor cells. Plasma concentrations of Trx-1 are up to 6 nM (6). In cells, Trx-1 is localized predominantly in the cytoplasm. Small amounts have been detected in the nucleus and in association with the outside surface of the cells. Expression of Trx-1 is increased under various stress conditions such as hypoxia, elevated hydrogen peroxide concentrations, photochemical oxidative stress, and viral and bacterial infections. Biological functions of Trx-1 include growth factor activity, antioxidant properties, a cofactor that provides reducing equivalents, and transcriptional regulation (1, 2). The synovial tissue of rheumatoid arthritis patients produces increased levels of Trx-1 under oxidative stress conditions, and a correlation exists between the plasma levels of Trx-1 and the severity of the disease, making Trx-1 a biomarker for this pathological condition (7, 8).

References: