Chaotropic or Buffer Tolerance

Buffer Name	Composition	Maximum concentration tolerated by assay	Effect at higher concentrations
Guanidine buffer	6 M GuHCl 0.1 M NaH2PO4 0.01 M Tris-HCl pH 8.0	5%	Inhibition
Urea buffer	7 M urea 0.1 M NaH2PO4 0.01 M Tris-HCl pH 8.0	5%	Inhibition
Gluthathione buffer	50mM Tris-HCL 50mM reduced Glutathione pH 8.0	0.1%	Inhibition
Mammalian detergent	Provided with the kit	50%	Inhibition
Yeast detergents	Commercially available	0.1%	Increase

Related Items/kits available from ADI (see details at the web site)

Catalog#	ProdDescription
800-420-GFP	Green Fluorescent Protein (GFP-fusion protein) ELISA Kit, 96 tests,
800-440-HIS	Histidine-tag (poly-His/Hisx6) Protein (His-tag-fusion protein) ELISA
720-100-GSG	Goat Anti-GST IgG (total) ELISA Kit, 2x 96 tests, Quantitative
720-110-GSR	Rabbit Anti-GST IgG (total) ELISA Kit, 2x 96 tests, Quantitative
720-120-GSC	Chicken Anti-GST IgG (total) ELISA Kit, 2x 96 tests, Quantitative
GST11-A	Anti-Glutathione Transferase (GST, E. coli) IgG# 1, aff pure
GST11-AP	Anti-Glutathione Transferase (GST, E. coli) IgG-AP conjugate
GST11-C	Recombinant purified Glutathione Transferase, GST (E. coli), WB +ve
GST11-HRP	alternate item # GST13-HRP; Rabbit Anti-Glutathione Transferase
GST11-R	Purified Recombinant Glutathione Transferase (E. coli) protein
GST11-S	Anti-Glutathione Transferase, GST (E. coli) antiserum # 1
GST12-M	Monoclonal Anti-Glutathione Transferase, GST (E. coli), ascites
GST13-A	Anti-Glutathione Transferase (GST, S. japonicum) IgG# 3
GST13-AS	Anti-Glutathione Transferase (GST, S. japonicum) IgG-Agarose (Aff
GST13-BTN	Anti-Glutathione Transferase (GST, S. japonicum) IgG-Biotin
GST13-FITC	Anti-Glutathione Transferase (GST, S. japonicum) IgG-FITC Conjugate
GST13-HRP	Anti-Glutathione Transferase (GST, S. japonicum) IgG-HRP Conjugate
GST13-R	Purified Recombinant Glutathione Transferase-His(x6) tag (GST-His)
GST14-R	Purified Recombinant Glutathione Transferase-Ubiquitin fusion (GST-
GST15R-AS	Glutathione Transferase (GST) Protein-Agarose (Aff matrix)
GST16-BTN	Monoclonal Anti-Glutathione Transferase (GST, E. coli) IgG-
GST16-M	Monoclonal Anti-Glutathione Transferase (GST, E. coli) IgG#2, purifed

Instruction Manual No. M-800-400-GST

Glutathione Transferase Protein (GST) or GST-fusion protein from S. Japonicum

ELISA KIT Cat. No. 800-400-GST

For Quantitative Determination of GST In cells or tissues

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

GST ELISA KIT Cat. No. 800-400-GST

Kit Contents: (reagents for 96 tests)

Components	
Anti-GST coated microwell **strip plate** (12x8 wells), #800421	1 plate
GST protein **Standard A** (200 ng) #800400A (**lyophilized**)	1 vial
GST-detection antibody **(10X), 0.7 ml** #800400AB	1 vial
Anti-GST-HRP **Conj (10X), 1 ml** #800422	1 vial
Sample Buffer, #800423, 15 ml	1 bottle
Incubation Buffer (10X), #800424, 6 ml	1 bottle
Wash Buffer (20X), #800425, 20 ml	1 bottle
HRP Substrate Solution; #800400HRP, 6 ml	1 bottle
Stop Solution #800420SS 11 ml	1 bottle
Complete Instruction **Manual**	M1830

Introduction

Expression of genes in E. coli or yeast or baculovirus offers a convenient system to produce large amounts of recombinant proteins that may otherwise be difficult to isolate from natural cells and tissues. Very often antibodies to these newly identified proteins are not available to study its biochemical properties, monitor protein expression, and purification. In order to circumvent this problem, short pieces of well-defined peptides (Poly-His, Flag-epitope or c-myc epitope or HA-tag) or small proteins (bacterial GST, MBP, Thioredoxin, b-Galactosidase, VSV-Glycoprotein etc) are often cloned along with the target gene. Proteins are expressed as fusion proteins. Antibodies to these fusion-tags are already available to monitor fusion protein expression and purification. Therefore, fusion-tags serve as universal tags much like secondary antibodies. Many tags have their own characteristics. Poly-His-fusion proteins (6 x His) can bind to Nickel-Sepharose or Nickel-HRP. GST-fusion proteins can bind to glutathione-Sepharose. Therefore, a high degree of purification of fusion protein can be achieved in just one affinity purification step. Purity of fusion proteins can be followed by Tag-antibodies. Very often, fusion proteins are directly injected into animals to generate antibodies. Some fusion tags can be removed later by treatment with enzymes to generate tag-free recombinant proteins.

GST is commonly used to create fusion proteins. The tag has the size of 220 amino acids (roughly 26 KDa), which, compared to other tags like the myc- or the FLAG-tag, is quite big. It is fused to the N-terminus of a protein. However, many commercially-available sources of GST-tagged plasmids include a thrombin domain for cleavage of the GST tag during protein purification. A GST-tag is often used to separate and purify proteins that contain the GST-fusion. GST-fusion proteins can be produced in Escherichia coli, as recombinant proteins.

GST or fusion protein expression can be detected by western or IHC but accurate quantitation for optimization of expression is difficult and time consuming. ADI's GST ELISA kit is a simple, rapid, and high throughput test for the measurement of GST or GST-fusion proteins in cells or tissues samples.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of GST detected using this assay is 1 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Five samples were run in ten replicates in an assay. The samples showed good intra-assay precision (4-8%CV).

Inter-assay precision:

Five samples were run in duplicate in eight independent assays. The samples showed good inter-assay precision (8-10 %CV).

3. Linearity of Dilution

A known amount of GST (1-25 ng/ml) was added to three HeLa cell extracts samples with initial GST of 9.5 ng/ml and the total GST concentrations measured. The assay showed excellent mean recoveries of about 95-115%).

4. Recovery

Sample Type	Average Recovery	(%) Range (%)
50% mammalian extraction buffer	94%	75 – 112%
5% Guanidine Buffer	79%	58 – 100%
5% Urea buffer	82%	71 – 93%
0.1% Gluthathione buffer	63%	47 – 79%
0.1% Yeast extraction buffers	91%	84 – 98%

If measuring GST tagged on proteins purified by gluthathione resins, dilute standard curve in 0.1% glutathione buffer and dilute eluate by 1:1000 factor. Gluthathione buffer can decrease significantly the sensitivity of the assay. It is recommended to dialyze the protein prior to assay measurement.

5. Specificity

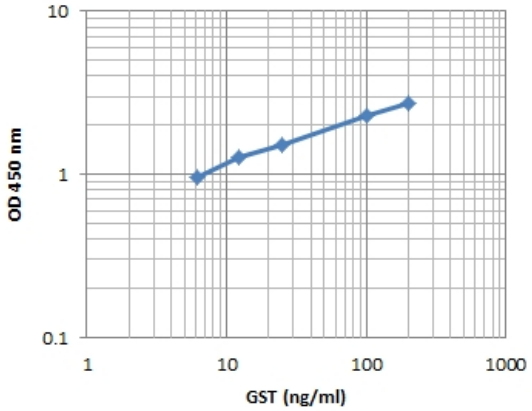
This kit is reactive with Schistosoma japonicum GST fused to His-tag at N-terminus and it was tested with recombinant full length protein GST-p53 (N terminal GST tag). Note: it is possible that improper folding of GST or the presence of a fusion partner at the C-terminus may prevent GST binding in this kit for other GST-Fusion proteins. The antibodies used in the kit reacted with purified GST or fusion proteins by Western blot.

References: Sheehan D (2001) Biochem. J. 360, 1-16; Oakley AJ (2005) Current Opinion in Structural Biology 15 (6): 716–23; Allocati N (2009) FEBS Journal 276 (1): 58–75; Hyes JD (2005) Annual Review of Pharmacology and Toxicology 45: 51–88

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Net Mean $A_{450\ nm}$	Calculated Conc. (U/ml)
A1, A2	**Std. A** (200)		
B1, B2	**Std. B** (100 ng/ml)		
C1, C2	**Std. C** (50 ng/ml)		
D1, D2	**Std. D** (25 ng/ml)		
E1, E2	**Std. E** (12.25 ng/ml)		
F1, F2	**Std. F** (6.12 ng/ml)		
F1, F2	**Blanks** (0 ng/ml)		
G1, G2	**Sample 1**		

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



*Kit-Spec-XL

A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Average the duplicate standard readings and plot against their concentrations after subtracting the zero standard reading. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Read GST protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in 1X Incubation buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

PRINCIPLE OF THE TEST

GST ELISA kit is a solid phase ELISA. The wells are coated with specific antibody to GST. The samples, std., and controls, and anti-GST detection antibody are allowed to bind to coated plates. Unbound GST is removed by washing the wells with buffer. Antibody-Enzyme conjugate is then added to all wells. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (blue color) is directly proportional to the amount of GST present in the sample. Adding stopping solution terminates the reaction and converts blue color into yellow. Absorbance is then measured on an ELISA reader at 450 nm. and the concentration of GST in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

This ELISA test is intended for *in vitro research* use only. The reagents contain proclin-300 as preservative; necessary care should be taken when disposing solutions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), HCl (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SAMPLE PREPARTION

Sample buffer does not contain any protease or phosphatase inhibitors. We recommend adding these inhibitors (not supplied) if working with cell or tissue lysates. For GST-fusion proteins in culture media or in semi or purified proteins, protease inhibitors may be omitted.

Mammalian Cell Lysates:

1. Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 10 min at 4oC.
2. Rinse cells twice with PBS. Solubilize cell pellet at $2x10^7$ cells/ml in sample buffer. .
3. Incubate on ice for 20 minutes. Centrifuge at 16,000x g, 4°C for 20 minutes. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay and samples adjusted to contain similar protein concn.

Bacterial and Yeast Cell Lysates

1. Prepare cell or tissue lysates by sonication or freeze thaw. Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

2. Clarify lysates by centrifuging at 16,000 x g, 4°C for20 minutes. If fusion protein is insoluble, use either 6 M Guanidine hydrochloride; 0.1 M NaPI, Tris-HCl, pH 8.0 or 7M urea buffers.

3. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

Sub-cellular organelle lysates e.g. mitochondria:

1. Prepare the organelle sample by, for example, subcellular fractionation.

2. Pellet the sample. Solubilize the pellet by adding 9 volumes Extraction Buffer.

3. Incubate on ice for 20 minutes. Centrifuge at 16,000 x g, 4°C for 20 minutes. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. The sample should be diluted to within the working range of the assay in 1X Incubation Buffer. As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Data Analysis.

REAGENTS PREPARATION

1. **Prepare 1x Wash buffer** by diluting 1:20 with distilled water (20 ml into 380 ml H2O). Store unused buffer at 4oC.

2. **Prepare 1x incubation buffer** by diluting 1:10 with 1X wash buffer (1 ml stock buffer into 5 ml 1x wash buffer. Store unused stock or 1x buffer at 4oC.

3. **Prepare 1x Antibody-HRP Conj** by diluting 1:10 with 1x incubation (100 ul stock into 900 ul 1x incubation buffer). Prepare in required amounts only (prepare 0.5 ml for each strip or 5 ml for full plate). Store unused buffer at 4oC.

Reconstitution and Preparation of GST Standards

1. Reconstitute the 2000 ng (2 ug) lyophilized standards in 200 ul of incubation 1X incubation buffer and gently mix for 10 min at room temp. This will be 10,00 ng/ml (10 ug/ml) stock. Make 150 ul aliquots of the stock store frozen at -20oC or below. Do not freeze and thaw the stock standards.
2. Prepare 200 ng/ml (Std A; dilute 20 ul of 10 ug/ml stock in 180 ul of 1X incubation buffer). Prepare Stds B-F using 2-fold serial dilution (150 ul of std and 150 ul of 1X incubation buffer), 100 ng/ml (Std B), 50 ng/ml (Std C), 25 ng/ml (Std D), 12.5 ng/ml (Std E) and 6.25 ng/ml (Std F) by serially diluting with 1X incubation buffer (example: Use freshly prepare stds A-F.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{O}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions.

HRP substrate solution should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use. Reconstituted control is stable for one week at 2-8^{O}C. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

1. Prepare 1X working buffers from stocks of wash buffer (1:20), blocking buffer (1:10) and anti-GST HRP Conjugate, and working standards from the stock vial of GST standards as suggested on page 2. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **50 ul of standards, control, and samples** into appropriate wells in *duplicate*. Use 1X incubation buffer for blanks or zero standards. **Incubate for 2 hrs at room temp**.
3. Remove incubation mixture and **wash the wells 2X** with wash buffer
4. Add **50 ul of detection antibody** into each well. Mix gently for 5-10 seconds and incubate for **60 min** at room temp.
5. Remove incubation mixture and **wash the wells 2X** with wash buffer.
6. Add **50 ul of anti-GST-HRP conjugate** into **each well.** Mix gently. Cover the plate and **incubate for 60 minutes** at room temperature. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
7. Remove reaction mixture and **wash 4X** with wash buffer.
8. Add **50 ul** of TMB substrate to al wells. Make sure that TMB solution is at room temp before adding to the plate. Mix the plate gently for 5-10 seconds. Cover the plate and incubate at room temp. for **15 minutes.** Blue color develops in standards and positive wells. Plates can be read at 600 nm or reaction stopped.
9. Stop the reaction by adding **50 ul of stop** solution to all wells**.** Mix gently for 5-10 seconds (blue color turns yellow).
10. Measure the absorbance at 450 nm using an ELISA reader within 30 min.

NOTES : Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{O}C.
Addition of the HRP substrate solution starts a kinetic reaction. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

samples containing more than highest standard GST should be diluted with the 1x incubation buffer and reassayed, and the results obtained should be multiplied by the appropriate dilution factor.