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

Catalog#	ProdDescription
800-400-GST	Glutathione Transferase (GST-fusion protein) ELISA Kit, 96 tests, Quantitative
800-420-GFP	Green Fluorescent Protein (GFP-fusion protein) ELISA Kit, 96 tests, Quantitative
800-440-HIS	Histidine-tag (poly-His/Hisx6) Protein (His-tag-fusion protein) ELISA Kit, 96 tests, Quantitative
GFP11-A	Anti-Green Fluorescent Proteins (GFP, A. victoria) protein, IgG
GFP12-M	Monoclonal Anti-Green Fluorescent Proteins (GFP, A. victoria) IgG
GFP11-HRP	Anti-Green Fluorescent Proteins (GFP, A. victoria) protein IgG-HRP conjugate
GFP11-AP	Anti-Green Fluorescent Proteins (GFP, A. victoria) protein IgG-Alk Phos (AP) conjugate
GFP11-BTN	Anti-Green Fluorescent Proteins (GFP, A. victoria) protein IgG-Biotin conjugate
GFP11-C	Green Fluorescent Proteins (GFP, A. victoria) protein for WB control
GFP15-R	Green Fluorescent Proteins (GFP) protein for ELISA or Standards
GFP11-FITC	Anti-Green Fluorescent Proteins (GFP, A. victoria) protein IgG-FITC conjugate
GFP15-R-100	Green Fluorescent Proteins (GFP) protein for ELISA or Standards
EGFP16-R	Enhanced Green Fluorescent Proteins (EGFP) protein for ELISA or Standards
EGFP16-R-100	Enhanced Green Fluorescent Proteins (EGFP) protein for ELISA or Standards
GFP12-AP	Monoclonal Anti-Green Fluorescent Proteins (GFP, A. victoria) protein IgG-Alk Phos (AP) conjugate
GFP12-BTN	Monoclonal Anti-Green Fluorescent Proteins (GFP, A. victoria) protein IgG-Biotin conjugate
GFP12-FITC	Monoclonal Anti-Green Fluorescent Proteins (GFP, A. victoria) protein IgG-FITC conjugate
GFP12-HRP	Monoclonal Anti-Green Fluorescent Proteins (GFP, A. victoria) protein IgG-HRP conjugate
MA-20144	Mouse Monoclonal Anti-Human Green fluorescence protein (GFP)

Instruction Manual No. M-800-420-GFP

Green Fluorescent Protein (GFP) or GFP-fusion protein

ELISA KIT Cat. No. 800-420-GFP

For Quantitative Determination of GFP In cells or tissues

For In Vitro Research Use Only

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GFP ELISA KIT Cat. No. 800-420-GFP
Kit Contents: (reagents for 96 tests)

Components	
Anti-GFP coated microwell **strip plate** (12x8 wells), #800401	1 plate
GFP protein **Standard A** (100 ng) #800402 (lyophilized(	1 vial
GFP-detection antibody **(10X), 0.7 ml** #800403	1 vial
Anti-GFP-HRP **Conj (10X), 1 ml** #800404	1 vial
Sample Buffer, #800405, 15 ml	1 bottle
Incubation Buffer (10X), #800406, 6 ml	1 bottle
Wash Buffer (20X), #800407, 20 ml	1 bottle
HRP substrate Solution; #800400T, 12 ml	1 bottle
Stop Solution #800400SS 11 ml	1 bottle
Complete Instruction **Manual**	M1830

Introduction

The green fluorescent protein (GFP) is a protein composed of 238 amino acid residues (26.9kDa) that exhibits bright green fluorescence when exposed to ultraviolet blue light. Although many other marine organisms have similar green fluorescent proteins, GFP traditionally refers to the protein first isolated from the jellyfish Aequorea victoria (excitation max at 395 nm and a minor one at 475 nm; emission at 509 nm, which is in the lower green portion of the visible spectrum). The fluorescence quantum yield (QY) of GFP is 0.79. In cell and molecular biology, the GFP gene is frequently used as a reporter of expression. In modified forms it has been used to make biosensors, and many animals have been created that express GFP as a proof-of-concept that a gene can be expressed throughout a given organism. The GFP gene can be introduced into organisms and maintained in their genome through breeding, injection with a viral vector, or cell transformation. To date, the GFP gene has been introduced and expressed in many Bacteria, Yeast and other Fungi, fish (such as zebrafish), plant, fly, and mammalian cells, including human.

GFP expression can be detected by fluorescence emission in cells and tissues but accurate quantitation for optimization of expression is difficult and time consuming. ADI's GFP ELISA kit is a simple and rapid test for the measurement of GFP in cells or tissues samples.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of human GFP detected using this assay is 25 pg/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 GFP (1-25 ng/ml) was added to three HeLa cell extracts samples with initial GFP of 9.5 ng/ml and the total GFP concentrations measured. The assay showed excellent mean recoveries of about 95-115%).

4. Recovery

Samples were extracted in in 50% extraction buffer and cell culture media with a recovery of GFP 80-105%.

5. Specificity

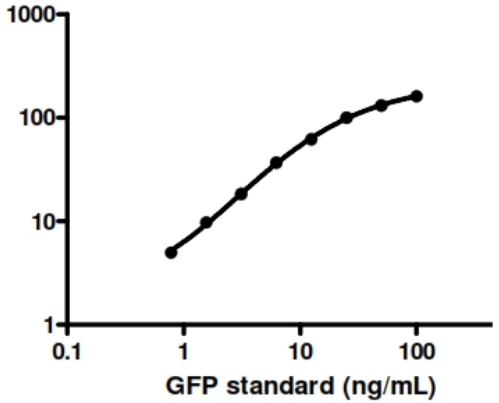
This kit is reactive with Aequorea victoria GFP. It is not tested with fluorescent protein variants (RFP, YFP, CFP, etc). Also note it is possible that improper folding of GFP or the presence of a fusion partner may prevent GFP binding in this kit.

References: Narayanan, S.R., J. Chromatogr., 658, 237 (1994); Olins, P.O., and Lee, S.C., Curr. Opin. Biotechnol., 4, 520 (1993); Uhlen, M., and Moks, T., Meth. Enzymol., 185, 129, (1990). Tsien, R.Y., Ann. Rev. Biochem., 67, 509 (1998); Chalifie, M., et al., Science, 263, 802 (1994); Cubitt, A.B., et al., Trends Biochem., 20, 448, (1995); Prasher, D.C., Trends Genet., 11, 320 (1995); Stearns, T., Curr. Biol., 5, 262 (1995); Errampalli, D., et al., J. Microbiol. Methods, 35, 187 (1999); Sawin, K.E., Methods Cell Biol., 58, 123 (1999); Cormack, B., Curr. Opin. Microbiol., 1, 406 (1998); Brand, A., Methods Cell Biol., 58, 165 (1999)

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Net Mean $A_{450\ nm}$	Calculated Conc. (U/ml)
A1, A2	**Std. A** (100)		
B1, B2	**Std. B** (50 ng/ml)		
C1, C2	**Std. C** (25 ng/ml)		
D1, D2	**Std. D** (12.5 ng/ml)		
E1, E2	**Std. E** (6.25 ng/ml)		
F1, F2	**Std. F** (3.12 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.



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 GFP 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

GFP ELISA kit is a solid phase ELISA. The wells are coated with specific antibody to GFP. The samples, std., and controls, and anti-GFP detection antibody are allowed to bind to coated plates. Unbound GFP 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 GFP 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 GFP 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 GFP-fusion proteins in culture media or in semi or purified proteins, protease inhibitors may be omitted.

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.

Tissue Lysates

1. 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. Suspend the homogenate to 25 mg/mL in PBS. Solubilize the homogenate by adding 4 volumes of Extraction Buffer to a sample protein concentration of 5 mg/mL.
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.

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 GFP Standards

1. Reconstitute the 100 ng lyophilized standards in 1 ml of incubation 1X incubation buffer. Add 1 ml of buffer and gently mix for 10 min at room temp. This will be 100 ng/ml stock (Std A). Make 150 ul aliquots of the stock store frozen at -20oC or below. Do not freeze and thaw the stock standards.
2. Prepare 50 ng/ml (Std B), 25 ng/ml (Std C), 12.5 ng/ml (Std D), 6.25 ng/ml (Std E), 3.12 ng/ml (Std F) by serially diluting with 1X incubation buffer (example: 150 ul of 100 ng/ml std and 150 ul 1x incubation buffer=50 ng/ml GFP). Use freshly prepare stds B-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-GFP HRP Conjugate, and working standards from the stock vial of GFP 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*. **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-GFP-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 100 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 100 ng/ml of GFP should be diluted with the 1x incubation buffer and reassayed, and the results obtained should be multiplied by the appropriate dilution factor.