GFP ELISA Kit

Enzyme Immunoassay for the quantification of Green fluorescent protein (GFP) or GFP fusion protein in cell culture supernatants, cell lysate and tissue lysate.

Catalog number: ARG82206

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INTRODUCTION

The green fluorescent protein (GFP) is a protein composed of 238 amino acid residues (26.9 kDa) that exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. Similar proteins that also fluoresce green are found in many marine organisms, but the label GFP traditionally refers to this particular protein, which was first isolated from the jellyfish Aequorea victoria and is sometimes called—when such precision is required—avGFP.

The GFP from A. victoria has a major excitation peak at a wavelength of 395 nm and a minor one at 475 nm. Its emission peak is at 509 nm, which is in the lower green portion of the visible spectrum. In cell and molecular biology, the GFP gene is frequently used as a reporter of expression. It has been used in modified forms to make biosensors, and many animals have been created that express GFP, which demonstrates a proof of concept that a gene can be expressed throughout a given organism, in selected organs, or in cells of interest. GFP can be introduced into animals or other species through transgenic techniques, and maintained in their genome and that of their offspring. [Wikipedia: Green fluorescent protein]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique for detection and quantitation of GFP or GFP fusion protein in cell or tissue samples. The quantity of GFP or its variants (including BFP, CFP and YFP) in an unknown sample is determined by comparing its absorbance with that of a known recombinant GFP standard curve. The kit also provides an efficient system for rapid quantitation of GFP lentivirus titer for both viral supernatant and purified virus. An antibody specific for GFP has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any GFP protein present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for GFP is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of GFP bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of GFP in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, the Standard should be aliquoted and stored at -80°C to avoid repeated freeze-thaw cycles. Store all other components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
antibody-coated microplate	12 X 8 strips	4°C
Standard (0.5 µg/ml)	100 µl	-80°C
10X Wash Buffer	100 ml	4°C
1000X Biotin conjugated-GFP Antibody concentrate	20 µl	4°C
2000X HRP-Streptavidin concentrate	20 µl	4°C
Assay Diluent	50 ml (Ready-to-use)	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620 nm as reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- Microplate shaker.
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, the Standard should be aliquoted and stored at -80°C to avoid repeated freeze-thaw cycles. Store all other components at 4°C.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1000 x g. Collect supernatant and assay immediately or aliquot and store samples at-80°C. Avoid repeated freeze-thaw cycles.

Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect samples and assay immediately or aliquot and store samples at-80°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer, mix well. Storage at 2-8°C.
- 1X Biotin conjugated-GFP Antibody working solution: Dilute the antibody immediately before use; dilute the 1000X Biotin-conjugated-GFP Antibody concentrate into Assay Diluent to yield 1X Conjugated antibody working solution. Do not store diluted solutions.
- 1X HRP-Streptavidin working solution: Dilute the reagent immediately before use; dilute the 2000X HRP-Streptavidin concentrate into Assay Diluent to yield 1X HRP-Streptavidin working solution. Do not store diluted solutions.
- Sample: If the assay found samples contain GFP higher than the highest standard (2000 pg/ml), the samples can be diluted with Assay Diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

(It is recommended making series dilutions with Assay Diluent for each unknown sample to do pre-test to determine the suitable dilution factor).

- **GFP standard:** Prepare a series dilution of GFP standards with Assay Diluent. The Assay Diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	GFP (pg/ml)	Assay Diluent (µl)	Standards (µl)
S1	2000	996	4 (0.5 µg/ml stock)
S2	1000	250	250 µl (S1)
S3	500	250	250 µl (S2)
S4	250	250	250 µl (S3)
S5	125	250	250 µl (S4)
S6	62.5	250	250 µl (S5)
S7	31.25	250	250 µl (S6)
S0	0	250	0

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

Warm Substrate Solution to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Standards and samples should be assayed in duplicates.
2. Add **100 µl of the Standards** and **samples** into the appropriate wells. Incubate for **2 hours at 37°C or overnight at 4°C.**
3. Aspirate each well and wash, repeating the process 2 times for a total **3 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting
4. Add **100 µl** of the **diluted Biotin-conjugated-GFP antibody working solution** to each well, incubate for **2 hours at RT** on a microplate shaker.
5. Aspirate each well and **wash as step 3**.
6. Add **100 µl** of the **diluted HRP-Streptavidin working solution** to all wells and incubate for **1 hour at RT** on a microplate shaker.
7. **Warm TMB substrate solution to RT** before next wash step.
8. Aspirate each well and **wash as step 3**. Proceed immediately to the next step.
9. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **2-30 mins at RT** on microplate shaker. Avoid exposure to light.
   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

10. Add 100 µl of Stop Solution to each well. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to yellow.
11. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

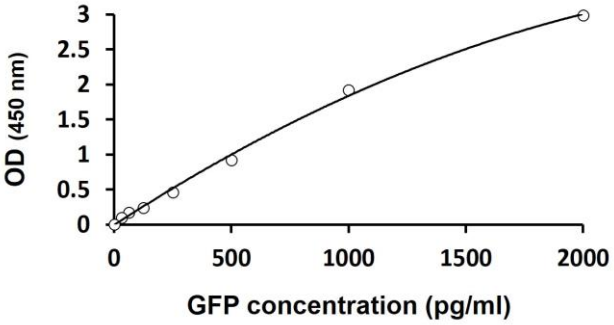
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

30 pg/ml

Specificity

GFP ELISA Kit will detect GFP, BFP, CFP, and YFP from Aequorea victoria.

Assay Range

30 –2000 pg/ml