Product Manual

GFP Quantitation Kit, Fluorometric

Catalog Number

AKR-120 100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Green Fluorescent Protein (GFP) is a spontaneously fluorescent protein originally isolated from the jellyfish *Aequorea victoria*. Molecular cloning of the GFP gene and its subsequent expression in heterologous systems have established recombinant GFP (rGFP) as a valuable reporter molecule for *in vivo* visualization of gene expression events in a wide variety of cell types and organisms. Since rGFP requires no additional substrates or cofactors, rGFP fluorescence can be easily detected under fluorescence microscope after expression in either prokaryotic or eukaryotic cells.

Most imaging studies of rGFP are qualitative. Quantitative analysis of rGFP level in cells by FACS is time consuming and expensive. Cell Biolabs' GFP Quantitation Kit measures GFP fluorescence in a fluorometer. The quantity of rGFP in sample is determined by comparing its fluorescence reading with that of known recombinant GFP standard curve. The kit has detection sensitivity limit of 100 ng/mL. A proprietary GFP quench solution is also included for determining autofluorescence of cell or tissue sample. Each kit provides sufficient reagents to perform up to 100 assays including standard curve and GFP samples.

Related Products

1. AKR-121: GFP ELISA Kit
2. AKR-122: RFP ELISA Kit

Kit Components

1. 5X Assay/Lysis Buffer (Part No. 240102): One 30 mL bottle
2. Assay Diluent (Part No. 212001): One 30 mL bottle
3. 10X GFP Quench Solution (Part No. 212002): One 12 mL bottle.
4. GFP Standard (Part No. 212003): One 20 µL vial of purified recombinant GFP at 1 mg/mL in 1X PBS

Materials Not Supplied

1. Cells or tissues expressing GFP or GFP fusion protein
2. Heating block
3. 96-well plate suitable for a fluorescence plate reader
4. Fluorescence plate reader

Storage

Store all kit components at 4ºC.

Preparation of Reagents

- 1X Assay/Lysis Buffer: Mix the 5X Stock briefly and dilute to 1X in deionized water. Just prior to usage, add protease inhibitors such as 1 mM PMSF, 10 µg/mL leupeptin, and 10 µg/mL aprotinin.

- 10X GFP Quench Solution: If precipitated crystals are present, briefly heat the solution at 37°C to redissolve the crystals.

Preparation of GFP Standard

1. Prepare a 10 µg/mL GFP standard solution by diluting the Recombinant GFP Standard stock (1.0 mg/mL) in Assay Diluent, for example, add 2.5 µL of the recombinant GFP standard to 247.5 µL of Assay Diluent.
2. Prepare a dilution series of recombinant GFP standards in 96-well plate suitable for a fluorescence plate reader in the concentration range of 0 ng/mL – 5 ng/mL in Assay Diluent (Table 1).

Standard Wells	10 µg/mL Recombinant GFP Standard (µL)	Assay Diluent (µL)	GFP (ng/mL)
1	100	0	10,000
2	50	50	5,000
3	25	75	2,500
4	12.5	87.5	1,250
5	6.3	93.7	625
6	3.1	96.9	313
7	1.6	98.4	156
8	0	100	0

Table 1. Preparation of samples for GFP Standard Curve

3. Briefly mix the standards and read the fluorescence with a fluorescence plate reader at 488 nm/507 nm.

Assay Protocol

1. Prepare cell or tissue lysates containing GFP or GFP fusion protein in 1X Assay/Lysis Buffer.
2. Transfer 100 µL of the GFP lysate sample to a 96-well plate suitable for fluorescence measurement. Read the fluorescence with a fluorescence plate reader at 488 nm/507 nm. Each GFP sample, blank, and control lysate should be assayed in duplicate.

Autofluorescence Measurement (optional)

1. Transfer 180 µL of the GFP sample to an eppendorf tube. Add 20 µL of 10X GFP Quench Solution. In a heating block, incubate the sample at 65ºC for 15 minutes.
2. Cool the solution to 37ºC or room temperature in a water bath for 5 minutes.
3. Centrifuge 5 minutes at 10,000 g and transfer 100 µL of the supernatant to a 96-well plate suitable for fluorescence measurement. Read the fluorescence with a fluorescence plate reader at 488 nm/507 nm.

Example of Results

The following figures demonstrate typical results with the GFP fluorometric quantitation Kit. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

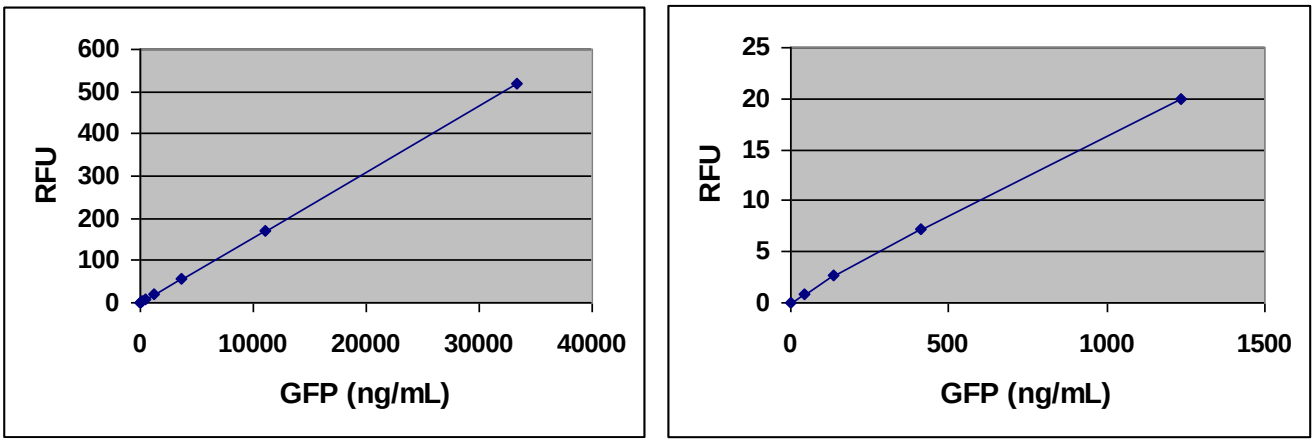

Figure 1. Recombinant GFP Standard Curve.

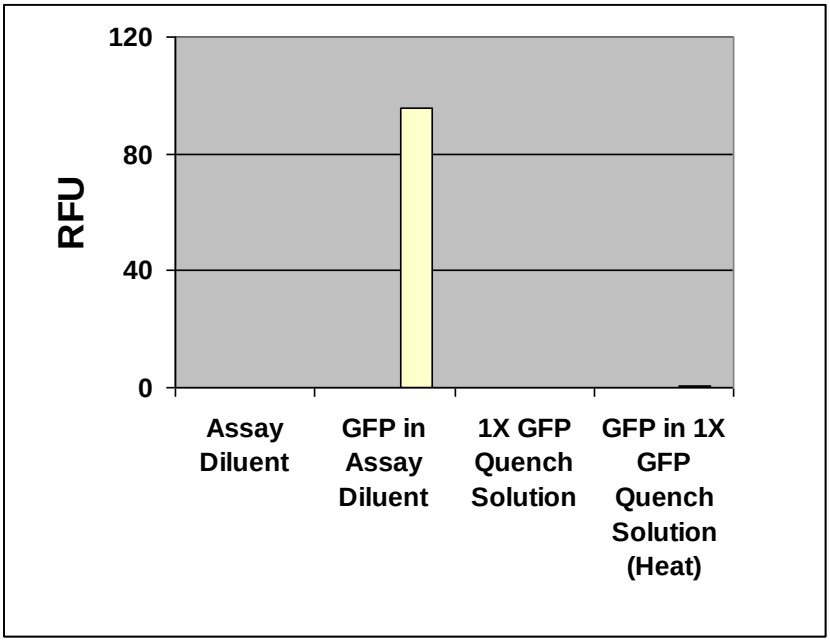

Figure 2. Inactivation of GFP by Heat and GFP Quench Solution.

Recent Product Citations

1. Occhialini, A. et al. (2020). Generation, analysis, and transformation of macro-chloroplast Potato (Solanum tuberosum) lines for chloroplast biotechnology. *Sci Rep*. **10**(1):21144. doi: 10.1038/s41598-020-78237-x.

2. Ahn, G. et al. (2019). Controlling the Release Profile Through Phase Control of Calcium Phosphate-Alginate Core-shell Nanoparticles in Gene Delivery. *Macromolecular Research*. doi:10.1007/s13233-019-7106-4.
3. Liu, J. et al. (2019). Human adenovirus type 17 from species D transduces endothelial cells and human CD46 is involved in cell entry. *Sci Rep*. **8**(1):13442. doi: 10.1038/s41598-018-31713-x.
4. Nichols, N. N. et al. (2018). Use of green fluorescent protein to monitor fungal growth in biomass hydrolysate. *Biology Methods and Protocols*. **3**(1). doi:10.1093/biomethods/bpx012.
5. Lipták, N. et al. (2018). Glomerulosclerosis in transgenic rabbits with ubiquitous Venus protein expression. *Acta Vet Hung*. **66**(2):281-293. doi: 10.1556/004.2018.026.
6. Shim, M. S. et al. (2014). Stimuli-responsive siRNA carriers for efficient gene silencing in tumors *via* systemic delivery. *Biomater Sci*. **2**:35-40.
7. Pfeiffer, B. et al. (2012). Using translational enhancers to increase transgene expression in Drosophila. *PNAS*. **109**:6626-6631.
8. Wamboldt, Y. et al. (2009). Participation of leaky ribosome scanning in protein dual targeting by alternative translation initiation in higher plants. *Plant Cell* 10.1105/tpc.108.063644.

Warranty

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