Product Information

GFP Quantitation Kit

I. Kit Contents:

Components	K2186-1000 1000 assays	Cap Color	Part Number
GFP Assay Buffer	25 ml	WM	K2186-C-1
GFP Standard (100 µg)	Lyophilized	Blue	K2186-C-2
GFP Quench Solution	1 ml	Red	K2186-C-3

II. Introduction:

Green Fluorescent Protein (GFP) and recombinant modified and enhanced GFP (EGFP) have been widely used in various biological experiments. Quantitative analysis of GFP expression has wider application than the qualitative ones in cell and tissues samples. This GFP Quantitation Kit quantifies GFP in a 96 micro-plate form. Cells and tissues can be directly homogenized in the GFP Assay Buffer. The quantity of GFP is measured via comparing its fluorescence with the GFP standard. The GFP concentration range of detection is 0.01 - 10 µg/ml. A GFP quench solution is also provided for determining auto-fluorescence of cell or tissue extracts.

III. Reagent Preparation:

GFP Standard: Reconstitute GFP Standard with 100 µl GFP Assay Buffer to generate 1 µg/µl GFP Standard Solution. Aliquot & store at - 20℃.

IV. Assay Protocol:

1. Standard Curve: Dilute 10 µl of the 1 µg/µl GFP Standard into 990 µl Assay Buffer to generate a 10 ng/µl working solution. Add 0, 8, 16, 24, 32, 40 µl into a 96 well plate in duplicate, bring the volume to 100 µl with GFP Assay Buffer to generate 0, 80, 160, 240, 320, 400 ng/well GFP standard.

Note: If a more sensitive assay is desired, the GFP standard working solution can be further dilute 10 fold to generate 0, 8, 16, 24, 32, 40 ng/well GFP standard curve.

2. Sample Extraction: Liquid samples can be assayed directly. For cells or tissues, 10^6 cultured cells or 50 mg tissues can be homogenized with 0.25 ml of assay buffer, incubate on ice for 10 min to ensure all the cells are lysed completely. Centrifuge 5 min at top speed. Transfer the clear supernatants to new tubes, store at -20℃.

3. GFP Quantification: Add 1 - 100 µl samples into 96 well plate, bring the volume to total 100 µl with Assay Buffer. For unknown samples, we suggest to assay several different doses to ensure the readings are within the standard curve. Read the samples and standards on a fluorescence microplate reader Ex/Em = 488/507 nm. Autofluorescence background (optional): Some tissue or cell extracts may contain significant amount of fluorescence. You may measure the autofluorescence by adding 20 µl of the GFP Quench Solution (if precipitation occurs in the solution, warm before use) into 180 µl samples in microtubes, mix and incubate at 65℃ on heating block for 10 min to quench GFP fluorescence, then measure the autofluorescence. The autofluorescence value should be subtracted from GFP readings.

4. Calculations: Subtract the 0 GFP Standard fluorescence reading from all samples and standards. Plot the GFP Standard Curve. Apply the sample fluorescence readings to the Standard Curve to get the GFP amount (A) in the sample wells.

GFP Concentration = A/V, ng/µl, or µg/ml

Where: A is GFP amount from standard curve (in ng).

V is sample volume added into the sample wells (in µl).

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com