GFP Quantitation Kit

Catalog Number KA0911

100 assays

Version: 04

Intended for research use only

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Introduction

Background

Green Fluorescent Protein (GFP) is a spontaneously fluorescent protein originally isolated from the jellyfish Aequorea Victoria (Chalfie et al, 1994). GFP has been recombinant modified and enhanced (called EGFP). GFP and EGFP have been widely used as a valuable reporter molecule for in vivo visualization of gene expression events in a variety of cell types and organisms. Since GFP requires no additional substrates or cofactors, GFP fluorescence can be easily detected under fluorescence microscope. However, most imaging studies of GFP are only qualitative. Since quantitative analysis of GFP expression level in cells or tissues are more informative and have wide applications. Abnova has developed the GFP Quantification Kit which quantifies GFP in 96 micro-plate format. Cells or tissues can be homogenized directly in the GFP Assay Buffer. The quantity of GFP is determined by comparing its fluorescence with that of GFP standard. The kit can detect a wide range of GFP concentration (0.01-10 µg/ml). A GFP quench solution is also provided for determining auto-fluorescence of cell or tissue extracts. Each kit provides sufficient reagents to perform up to 100 assays, Ex/Em = 488nm/507nm.

General Information

Materials Supplied

List of component

Component	Amount
GFP Assay Buffer	25 ml
GFP Standard (100 µg)	lyophilized
GFP Quench Solution	1 ml

Storage Instruction

Store kit at -20℃.

Assay Protocol

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℃.

Assay Procedure

- Standard Curve

  Dilute 10 µl of the 1 µg/µl GFP Standard into 990 µl Assay Buffer to generate 10 ng/ul working solution. Add 0, 8, 16, 24, 32, 40 µl* into 96 well plate in duplicates, 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.*
- 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℃.
- GFP Quantification

  Add 1-100ul 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 micro-plate reader Ex/Em = 488nm/507nm.

  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 up before use) into 180 µl samples in micro-tubes, 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.

Data Analysis

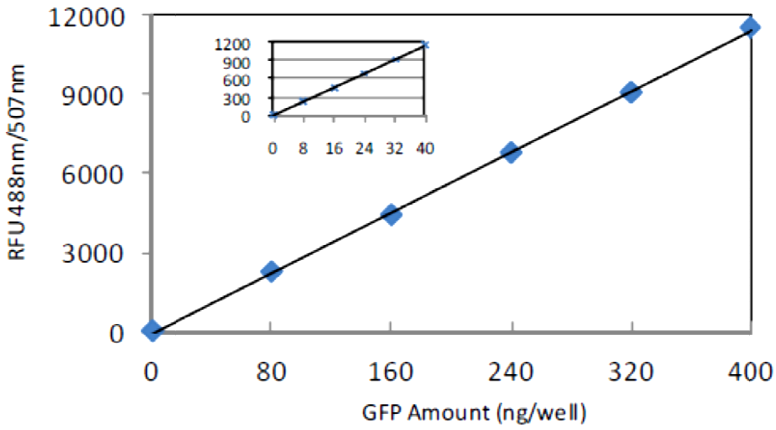
Calculation of Results

Subtract the 0 GFP 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).



GFP Standard Curve: The Assay was performed follow the kit procedure.