GFP Reporter Assay Kit

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K815
Description	GFP (Green Fluorescent Protein) is a fluorescent protein originally isolated from the jellyfish Aequorea victoria. GFP and its enhanced variant EGFP have been widely used as reporter proteins for in vivo monitoring of gene expression in a variety of cell types and organisms. Since GFP requires no additional substrates or cofactors, GFP fluorescence can easily be detected using a fluorescence microscope. However, most imaging studies of GFP are qualitative. Quantitative analyses of GFP expression levels in cells or tissues are more informative and have wider applications. The GFP Reporter Assay Kit has been designed for use in a 96 micro-plate format (Ex/Em = 488 nm/507nm). Cells or tissues can be homogenized directly in the GFP Assay Buffer. The amount of GFP is determined by comparing its fluorescence with that of a GFP standard, and the kit can detect a wide range of GFP concentrations (0.01-10 µg/ml). A GFP quench solution is also provided for determining autofluorescence levels of cell or tissue extracts.
Quantity	100 assays
Kit Components	Components: GFP Assay Buffer — Quantity: 25 ml — Cap-Code: NM GFP Standard (1 µg/µl) — 100 µl — Blue Cap GFP Quench Solution — 1 ml — Red Cap
Applications / Assay Protocol	1. Standard Curve: Dilute 10 µl of the 1 µl/µl GFP Standard into 990 µl Assay Buffer to generate 10 ng/ul working standard 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. 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 minutes to ensure all the cells are lysed completely. Centrifuge 5 minutes at top speed. Transfer the clear supernatants to new tubes, store at –20°C. 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 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°C on heating block for 10 minutes to quench GFP fluorescence, then measure the autofluorescence. The autofluorescence value should be subtracted from GFP readings. 4. Calculations: 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).
Storage & Stability	Store kit at –20°C upon arrival. Store individual reagents as indicated on the respective labels.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

North America 1 – 866 – 251 – 2860 (toll free)
Deutschland 0800 – 776 66 23 (gebührenfrei)
France 0800 90 93 32 (ligne verte)
United Kingdom 0800 – 96 03 33 (toll free)
Other Countries +49 6221 – 649 34 0

Email: info@promokine.info
www.promokine.info