



GFP Quantification NIT (Catalog # K815-100; 100 assays; Store kit at -20 °C)

I. Introduction:

Green Fluorescent Protein (GFP) is a spontaneously fluorescent protein originally isolated from the jellyfish *Aequorea Victoria* (Chalfie et al, 1994). It has also been expressed as a recombinant protein and 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 the fluorescence microscope. However, most imaging studies of GFP are only qualitative. Since quantitative analysis of GFP expression level in cells or tissues is more informative and has wide applications, BioVision's has developed the **GFP Quantification Kit**, which quantifies GFP in 96 well-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 the GFP standard. The kit can detect a wide range of GFP concentration (0.01-10 µg/ml). A GFP quench solution is also provided with the kit for determining the auto-fluorescence of cell or tissue extracts. Each kit provides sufficient reagents to perform up to 100 assays, Ex/Em = 488/507 nm.

II. Kit Contents:

Components	K815-100	Cap Code	Part Number
GFP Assay Buffer	25 ml	WM	K815-100-1
GFP Standard (100 µg)	Iyophilized	Blue	K815-100-2
GFP Quench Solution	1 ml	Red	K815-100-3

III. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plate is preferred.
- Multi-well fluorescence plate reader.

IV. Reagent Preparation:

GFP Standard: Reconstitute GFP Standard with 100 μ I GFP Assay Buffer to generate 1 μ g/ μ I GFP Standard Solution. Divide into aliquots & store at -20 °C.

V. Assay Protocol:

 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 duplicates and adjust 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 diluted 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, 10⁶ cultured cells or 50 mg tissues can be homogenized in 0.25 ml of GFP 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 °C.
- GFP Quantification: Add 1 100 μl samples into a 96 well plate and bring the volume to total 100 μl with GFP 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.

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Autofluorescence background (optional): Some tissue or cell extracts may contain significant amount of fluorescence. You may measure the autofluorescence by adding 20 μ l

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 °C on heating block for 10 min to quench the GFP fluorescence and then measure the autofluorescence. The autofluorescence value should be subtracted from the 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 GFP Standard Curve to get the GFP amount (A) in the sample wells.

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



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

RELATED PRODUCTS:

- Fluorescence Proteins & Antibodies
- Green Fluorescence protein (GFP)
- Red Fluorescence protein (RFP, dsRed)
- Cyan Fluorescence protein (CFP)
- Yellow Fluorescence protein (YFP)
- Fluorescence protein Antibodies
- Fluorescence protein expression vectors
- Apoptosis Assay Kits and Reagents
- Metabolism Assay Kits
- Obesity Assay Kitts
- Cell Proliferation Assay Kits
- Cytotoxicity Assay Kits
- Cell Fractionation Kits
- Cell Damage and Repair Assay Kits
- Kinase & Phosphatase Assay Kits
- Protein A/G/L & Sepharose Conjugates
- Adipokines and Lipoproteins
- Growth Factors & Cytokines
- Quality Antibodies

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