Version 3a, Last updated 9 June 2025

ab235672
GFP Quantification Kit

For the measurement of GFP in a variety of cell and tissue samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

The GFP Quantification Kit (ab235672) quantifies GFP in a 96-microplate format for a wide array of cells and tissues.

This kit can detect a wide range of GFP concentrations (0.01-10 µg/ml). Cells or tissues can be homogenized directly in the GFP Assay Buffer, the quantity of GFP is then determined by comparing the fluorescence with that of the GFP standard.

Prepare all samples, standards and controls as directed.

Dilute the GFP standard with Assay Buffer to prepare the GFP standard curve.

Add desired amount of sample (1-100 µL) into the 96-well plate, bring the volume to total 100 µL with Assay Buffer.

Read the samples and standards on a fluorescence microplate reader (Ex/Em = 488/528 nm, 515 nm if cutoff filter is used).

Apply the sample fluorescence readings to the Standard Curve to get the GFP amount in the sample wells.

2. Materials Supplied and Storage

Store kit at -20°C immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
GFP Assay Buffer	25 ml	-20°C	-20°C
GFP Standard	1 vial	-20°C	-20°C
Stop Solution II	1.2 ml	-20°C	-20°C

PLEASE NOTE: Stop Solution II was previously labelled as GFP Quench Solution. The composition has not changed.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 488/528 nm, if a cutoff filter is used, it should be 515 nm.
- 96 well plate with opaque flat bottom, preferably black.
- Dounce homogenizer (if using tissue)

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 GFP Assay Buffer

1. Ready to use as supplied.

5.2 GFP Standard (100 µl)

1. Reconstitute GFP standard with 100 µL GFP Assay Buffer to generate 1 µg/µl GFP Standard Solution.
2. Aliquot and store at -20°C.

5.3 Stop Solution II

1. Ready to use as supplied.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare a 10 ng/µl GFP standard by adding 10 µL of the 1 µg/µl GFP standard into 990 µL Assay Buffer. Mix well.

2. Using the 10 ng/µl GFP standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	10 ng/µl Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount of GFP standard in well (ng/well)
1	0	200	100	0
2	16	184	100	80
3	32	168	100	160
4	48	152	100	240
5	64	136	100	320
6	80	120	100	400

Each dilution has enough standard to set up duplicate readings (2 x 100 µL).

ΔNote: 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.

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay. Include if samples can be frozen: If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware, however, that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.

7.1 Liquid samples:

1. Liquid samples can be assayed directly.

7.2 For cells or tissues:

1. 10^6 cultured cells or 50 mg tissues can be homogenized with 0.25 ml of assay buffer.
2. Incubate on ice for 10 minutes to ensure all the cells are completely lysed.
3. Centrifuge for 5 minutes at top speed.
4. Transfer the supernatants to new tubes and store at -20°C.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 GFP Quantification:

1. Add 1-100 µL samples into the 96-well plate, bring the volume up to total 100 µL with Assay Buffer.
2. For unknown samples, we suggest to assay several different doses to ensure the readings lie within the standard curve.
3. Read the samples and standards on a fluorescence microplate reader at Ex/Em = 488/528 nm. If a cutoff filter is used, it should be 515 nm.

8.2 Auto fluorescence background (optional):

Some tissue or cell extracts may contain a significant amount of fluorescence. You may measure the autofluorescence by adding 20 µl of the Stop Solution II (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 minutes to quench GFP fluorescence, then measure the autofluorescence. The autofluorescence value should be subtracted from GFP readings.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #0) from all standards, controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final concentration of GFP.
5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
6. Apply the corrected sample fluorescence readings to the standard curve to get GFP (B) amount in the sample wells.
7. Concentration of GFP in ng/µL in the test samples is calculated as:

$$\text{GFP concentration} = \frac{B}{V} * D$$

Where:

B = amount of GFP in the sample well calculated from standard curve in ng.

V = sample volume added in the sample wells µL.

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

10. Typical Data

Data provided **for demonstration purposes only**.

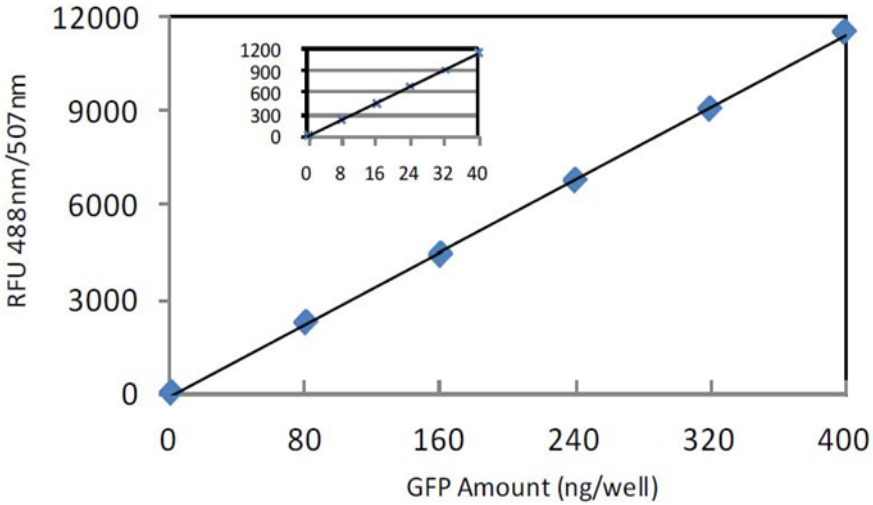

Figure 1: GFP standard curve. The assay was perfromed following the kit procedure.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)