



mVenus Fluorescence Standard Kit

Manual

Catalog #	5014
Package Size	100 Assays

Custom formats and package sizes are available upon request



Important!

4 °C Storage Required

- * Immediately inspect packages
- * Refrigerate upon receipt



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Description:

The IG® mVenus Fluorescence Standard Kit contains purified recombinant mVenus protein for standardizing and quantifying GFP/YFP fluorescence in biological samples. mVenus is a bright yellow fluorescent protein (YFP) widely used in live-cell imaging and other fluorescence-based applications because of its strong fluorescence and high quantum yield.

Following the kit procedure, the amount of mVenus in a sample can be determined by comparing its fluorescence intensity with a standard curve generated from known concentrations of recombinant mVenus. The kit is suitable for fluorescence plate readers, spectrofluorometers, confocal microscopes, and other compatible fluorescence-detection instruments.

With a standard fluorescence plate reader, the IG® mVenus Fluorescence Standard Kit provides a detection limit of approximately 100 ng/mL. A lower detection limit may be achieved with higher-sensitivity instruments, such as spectrofluorometers or confocal microscopes. The excellent thermal stability of mVenus also makes the standard suitable for a broad range of fluorescence assays, instrument calibration, assay development, and quality-control applications.

Applications:

- Quantification of mVenus and YFP fluorescence in biological samples
- Standardization of fluorescence from mVenus -transfected cells using FastAmp® Plant Cell Lysis Solution
- Calibration of spectrofluorometers and other fluorescence instruments for *in vitro* mVenus quantification
- Generation of recombinant mVenus standard curves
- Use as a positive control in fluorescence assays

Benefits:

- Enables reliable and reproducible mVenus/YFP quantification
- Supports instrument calibration and assay optimization
- Compatible with multiple fluorescence-detection platforms
- Provides a detection limit of approximately 100 ng/mL with standard fluorescence plate readers
- Thermostable and convenient for routine fluorescence assays

Source:

Recombinant mVenus expressed from a plasmid carrying the thermostable YFP gene.

Specifications:

- Purified recombinant mVenus protein
- Molecular weight: 31 kDa per monomer
- Free of detectable endonuclease activity

Components and Storage Temperature:

The following reagents are supplied with this product:

IG Cat #	Package Size	Component Name	IG Part #	Amount	Concentration	Storage
5014	100 Assays	mVenus	P241254	1 × 20 µL	1 mg/mL	4 °C
		FastAmp® Plant Cell Lysis Solution	P240378	1 × 12.5 mL	1X	4 °C
		Fluorescence Assay Buffer	P241250	1 × 30 mL	1X	4 °C

Custom formats and package sizes are available upon request.

Quality Control:

Protein Concentration: The stock protein concentration is determined by UV absorbance and confirmed by running against a standard using PAGE densitometry.

Fluorescence Quantum Yield: Protein dilutions and fluorescence measured 515 nm excitation and 530 nm emission match a mVenus batch control standard.

Preparation for mVenus Standard Curve:

This protocol is designed for use with a fluorescence plate reader. Sample volumes may be adjusted as needed for cuvettes or microscope specimen wells.

NOTE: A user may use their own sample buffer as needed. The series below uses the kit's assay buffer.

1. Prepare a 1 to 100 dilution of mVenus into Fluorescence Assay Buffer to make a 10 µg/mL working stock solution
2. Set up a dilution series into vials using the table below as a reference.

Standard #	10 µg/mL mVenus Working Stock Solution (µL)	Assay Buffer (µL)	Assay mVenus Concentration (ng/mL)
1	100	0	10,000
2	50	50	5,000
3	25	75	2,500
4	12.5	87.5	1,250
5	6.3	93.7	625
6	3.1	96.9	313
7	1.6	98.4	156
8	0	100	0

3. Briefly mix the standards and measure the fluorescence amplitude of each sample. Set the excitation to 550 nm and the emission to 583 nm for mVenus standard curve measurements.

Tissue Preparation with FastAmp® Plant Cell Lysis Solution (Optional):

1. Add 500 µL of 1X FastAmp® Plant Cell Lysis Solution to 50 mg of tissue in a 1.5 mL microcentrifuge tube. Prepare a blank control containing lysis solution without tissue.
2. Manually grind tissue with a micro-pestle for 30 seconds.
3. Centrifuge the samples in a benchtop centrifuge at maximum speed for 5 minutes.
4. Carefully transfer tissue extract supernatant to fresh 1.5 mL conical tubes
5. Transfer 100 µL for fluorescence intensity measurements using the same instrument settings used for the standard curve.
6. Compare the fluorescence of the sample blank with that of the standard-curve blank for background fluorescence.

Plot mVenus Standard Curve:

1. Plot fluorescence intensity (RFU) as a function of the Assay's mVenus Concentration provided in the table above. It is appropriate to subtract the background buffer from all standards and samples.
2. Fit the data points using linear regression, i.e. a line, to connect all data points. A good fitted curve will intersect all mVenus standard data points. The equation will have the form below:

$$\text{RFU} = \text{slope} \times [\text{mVenus}] + (\text{y-intercept})$$

3. Use the equation of the line to interpolate the sample's concentration of mVenus:

$$[\text{mVenus}] \text{ sample} = (\text{RFU} - \text{y-intercept})/\text{slope}$$

References:

1. Kremers, G.-J., Goedhart, J., van Munster, E. B. & Gadella, T. W. J. Cyan and Yellow Super Fluorescent Proteins with Improved Brightness, Protein Folding, and FRET Förster Radius,. *Biochemistry* 45, 6570–6580 (2006).

Related Products:

- IG® DsRed Fluorescence Standard Kit (Cat.# 5110)
- FastAmp® Plant Cell Lysis Solution (Cat.# 4611)
- IG® SYBR Green qPCR 2X Master Mix (Cat.# 3354)
- FastAmp® qRPA SYBR kit (Cat.# 3611)
- Cas9 Nuclease (Cat.# 3370)

Ordering Information:

- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
- Order by email, phone, or fax.
Email: **sales@intactgenomics.com**
Phone: (314) 942-3655 | Toll-free : 855-835-7172 | Fax: (314) 942-3656
- Order via our distributors.



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