



DsRed Fluorescence Standard Kit

Manual

Catalog #	5010
Package Size	100 Assays



Important!

4 °C Storage Required

- * Immediately inspect packages
- * Refrigerate upon receipt



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Intact Genomics, Inc.

DsRed Fluorescence Standard Kit

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Description:

The IG® DsRed Fluorescence Standard Kit contains purified recombinant DsRed protein for the quantitative measurement of DsRed and related red fluorescent protein signals in biological samples. DsRed is a red fluorescent protein commonly used in living-cell applications because of its strong and readily detectable fluorescence.

Using the procedure provided with the kit, the amount of DsRed in a sample can be determined by comparing its fluorescence intensity with a standard curve generated from known concentrations of recombinant DsRed. The standard is suitable for use with fluorescence microplate readers, spectrofluorometers, confocal microscopes, and other compatible fluorescence-detection systems.

The IG® DsRed Fluorescence Standard provides a detection sensitivity of approximately 500 ng/mL when used with a standard fluorescence microplate reader. Greater sensitivity may be achieved with spectrofluorometers, confocal microscopes, or other high-sensitivity detection instruments. The excellent thermal stability of DsRed also makes the standard suitable for a broad range of fluorescence assays, instrument verification, assay development, and quality-control applications.

Applications:

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

Benefits:

- Enables reliable quantification of DsRed fluorescence
- Improves consistency and reproducibility across assays
- Supports instrument calibration and standard curve generation
- Compatible with spectrofluorometers and other fluorescence instruments
- Thermostable and convenient for routine use

Source:

Recombinant DsRed expressed from a plasmid carrying the thermostable RFP gene.

Specifications:

- Purified recombinant DsRed protein
- Molecular weight: approximately 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
5010	100 Assays	DsRed	P241246	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

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 DsRed batch control standard.

Preparation for DsRed Standard Curve:

This protocol is for a plate reader but volumes may be adjusted for cuvettes or specimen wells under a microscope.

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 DsRed 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 DsRed Working Stock Solution (µL)	Assay Buffer (µL)	Assay DsRed 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 DsRed 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 DsRed Standard Curve:

1. Plot fluorescence intensity (RFU) as a function of the Assay's DsRed 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 DsRed standard data points. The equation will have the form below:

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

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

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

References:

1. Baird, G. S., Zacharias, D. A. & Tsien, R. Y. Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proceedings of the National Academy of Sciences* 97, 11984–11989 (2000).
2. Sacchetti, A., Subramaniam, V., Jovin, T. M. & Alberti, S. Oligomerization of DsRed is required for the generation of a functional red fluorescent chromophore. *FEBS Letters* 525, 13–19 (2002).

Related Products:

- IG® mVenus Fluorescence Standard Kit (Cat.# 5114)
- 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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