mCherry Quantification Kit (#BN01130)

(Catalog #BN01130; 100 assays; Store at -20°C)

I. Introduction:

mCherry is the second generation monomeric red fluorescent protein that was derived from proteins originally isolated from Cnidarians (jelly fish, sea anemones and corals), such as GFP or DsRed. mCherry is widely used as a fluorescent tracer in transfection and transgenic experiments because of its better performance in brightness, photostability and monomeric structure. Since mCherry requires no additional substrates or cofactors, mCherry can be easily detected under a fluorescence microscope. However, most imaging studies of mCherry are only qualitative. Assay Genie's mCherry Quantification Kit provides an easy 96 micro-plate assay to analyze mCherry expression level in cells or tissues quantitatively. Cells or tissues can be homogenized directly in the mCherry Assay Buffer. The amount of mCherry is determined by comparing its fluorescence (Ex/Em= 587/610 nm) with a mCherry standard that is provided in this kit. Each kit provides sufficient reagents to perform up to 100 assays.

II. Applications:

- Measurement of fluorescence of mCherry in tissue and cell lysates

III. Sample Type:

- Animal tissues: heart, liver, muscle, etc.
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	BN01130	Cap Code	Part Number
mCherry Assay Buffer	25 ml	WM	BN01130-1
mCherry Standard	1 vial	Red	BN01130-2

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom.
- Multi-well spectrophotometer (fluorescence reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **mCherry Assay Buffer:** Warm to room temperature before use. Store at 4°C or -20°C.
- **mCherry Standard:** Reconstitute with 100 µl dH_2O to generate 1 µg/ul. Aliquot and store at -20°C. Keep on ice while in use.

VII. mCherry Quantification Assay Protocol:

1. **Sample Preparation:** 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 mCherry Assay Buffer, followed by incubation on ice for 10 min. to ensure all the cells are lysed completely. Centrifuge samples at 10 x *g*, for 5 min. at 4 °C. Transfer the clear supernatants to new tubes. Store at -20°C and protect from light. Add 2-100 µl samples into a 96-well white plate. Adjust the volume to 100 µl with mCherry Assay Buffer. Mix well.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   b. Green Fluorescence Protein Quantification Kit is also available

2. **mCherry Standard Curve:** Dilute 10 µl of the 1 µg/µl mCherry Standard into 990 µl Assay Buffer to generate a 10 ng/µl mCherry working solution. Add 0, 2, 4, 6, 8 and 10 µl of mCherry working solution into a series of wells in a 96-well white plate to generate 0, 20, 40, 60, 80, 100 ng/well of mCherry Standard. Adjust the volume to 100 µl/well with mCherry Assay Buffer.

   Notes:

   a. If a more sensitive assay is desired, the mCherry working solution can be further diluted 10-fold to generate a 1 ng/µl mCherry working solution. Add 0, 2, 4, 6, 8 and 10 µl of mCherry working solution into a series of wells in a 96-well white plate to generate 0, 2, 4, 6, 8, 10 ng/well of mCherry Standard. Adjust the volume to 100 µl/well with mCherry Assay Buffer. Mix well.

3. **Measurement:** Measure fluorescence within 10 minutes at Ex/Em= 587/610 nm at room temperature.

4. **Calculation:** Subtract 0 mCherry Standard fluorescence reading from all readings. Plot the mCherry Standard Curve. Apply the fluorescence reading of samples to the mCherry Standard Curve to get the mCherry amount (A) in the sample wells.

Sample mCherry concentration = A/V x D (ng/µl)

Where: **A** = mCherry amount from Standard Curve (ng)
V = sample volume added into the reaction well (µl)
D = Dilution Factor

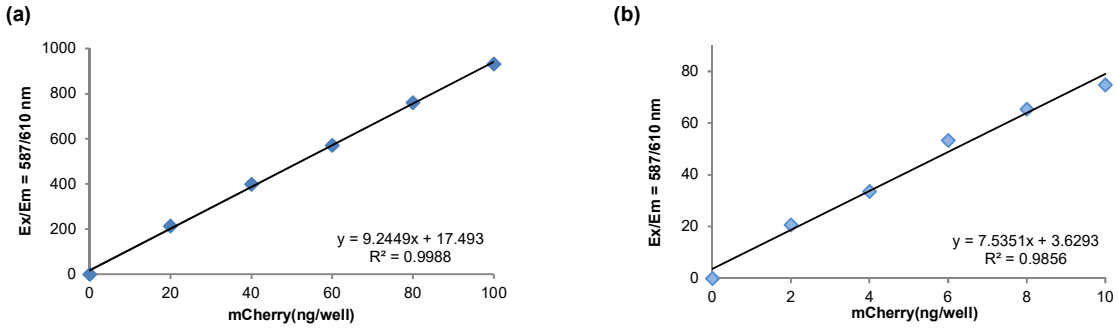

Figure: (a) mCherry Standard Curve for detection range between 0-100 ng/well. (b) mCherry Standard Curve for better sensitivity (0-10 ng/well) by using 1 ng/µl mCherry working solution to generate the standard curve. ΔRFU: relative fluorescence unit after subtraction of the buffer (0 mCherry) fluorescence reading.

FOR RESEARCH USE ONLY! Not to be used on humans.