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# Data Sheet

## *PRMT5 TR-FRET Assay Kit*

**Catalog # 52171**
**Size: 384 reactions**

**DESCRIPTION:** The *PRMT5 TR-FRET Assay Kit* is designed to measure PRMT5 activity in a homogeneous 384 reaction format. PRMT5 is a histone methyltransferase that exhibits methylation activity toward H4-R3. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The *PRMT5 TR-FRET Assay Kit* comes in a convenient format, with histone H4 peptide substrate, a Eu-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, FRET detection buffer, dye-labeled acceptor, and purified PRMT5 for 384 enzyme reactions. The key to the *PRMT5 TR-FRET Assay Kit* is a highly specific antibody that recognizes methylated substrate.

With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and methyltransferase enzyme for 120 minutes. Next, antibody is added. Finally, dye-labeled acceptor is added followed by fluorescence detection.

**COMPONENTS:**

| Catalog # | Component | Amount | Storage | |
|---|---|---|---|---|
| 51048 | PRMT5/MEP50 | 40 µg | -80°C | ***Avoid freeze/ thaw cycles!*** |
| 52120 | 60 µM S-adenosylmethionine | 250 µl | -80°C | |
| | Eu-labeled antibody | 5 µl | -80°C | |
| | Biotinylated histone H4 peptide substrate | 1000 rxns | -80°C | |
| | 4x PRMT5 assay buffer* | 3 x 1 ml | -20°C | |
| | Dye-labeled acceptor | 2 x 10 µl | -20°C | |
| | TR-FRET Detection Buffer | 4 ml | -20°C | |
| Fisher 07-200-330 | White, Nonbinding Corning, low volume, microtiter plate | 1 | Room temp. | |

* Add 10 µl of 0.5 M DTT per vial before use.

## MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

0.5M DTT (Dithiothreitol, Sigma Aldrich, Cat. #D0632)
Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)

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Adjustable micropipettor and sterile tips

**APPLICATIONS:** Great for screening small molecular inhibitors for drug discovery and HTS applications.

**STABILITY:** At least 6 months from date of receipt when stored as directed.

**REFERENCE:** Yang, Y., Bedford, M.T. 2013. *Nat Rev Cancer.* **13(1):**37-50.

**ASSAY PROTOCOL:**
***All samples and controls should be tested in duplicate.***

**Step 1:**

1) Re-suspend tube with **Biotinylated histone H4 peptide substrate** in 500 µL of distilled water.

2) Add 10 µl **0.5M DTT** (not provided) to a 1-ml tube of **4x PRMT5 Assay Buffer.** Prepare **1x PRMT5 Assay Buffer** by adding 1 part of **4x PRMT5 Assay Buffer** to 3 parts water (v/v). Dilute only enough **4x PRMT5 Assay Buffer** as required for the assay.

3) Thaw **S-adenosylmethionine** on ice. Upon first thaw, briefly spin tube containing **S-adenosylmethionine** to recover full content of the tube. Aliquot **S-adenosyl-methionine** into single use aliquots. Store remaining **S-adenosylmethionine** in aliquots at -80ºC immediately. *Note: S-adenosylmethionine is very sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

4) Prepare the master mixture: N wells × (2.5 µl **4× PRMT5 Assay Buffer** + 0.5 µl **60 µM S-adenosylmethionine** + 0.5 µl **Histone Substrate** + 1 µl **$H_2O$**)

5) Add 4.5 µl of master mixture to each well designated for the “Positive Control”, “Test Inhibitor”, and “Blank”. For the “Substrate Control”, add 2.5 µl **4× PRMT5 Assay Buffer** + 0.5 µl **Histone Substrate** + 1.5 µl **$H_2O$**.

6) Add 3 µl of inhibitor solution of each well designated “Test Inhibitor”. For the “Positive Control”, “Substrate Control” and “Blank”, add 3 µl of the same solution without inhibitor (inhibitor buffer).

7) Add 2.5 µl of **1 × PRMT5 assay buffer** to the well designated “Blank”.

| | Blank | Substrate Control | Positive Control | Test Inhibitor |
|---|---|---|---|---|

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| | | | | |
|---|---|---|---|---|
| 4× PRMT5 assay buffer | 2.5 µl | 2.5 µl | 2.5 µl | 2.5 µl |
| 60 µM S-adenosylmethionine | 0.5 µl | – | 0.5 µl | 0.5 µl |
| Histone substrate | 0.5 µl | 0.5 µl | 0.5 µl | 0.5 µl |
| $H_2O$ | 1 µl | 1.5 µl | 1 µl | 1 µl |
| Test Inhibitor/Activator | – | – | – | 3 µl |
| Inhibitor buffer (no inhibitor) | 3 µl | 3 µl | 3 µl | – |
| 1× PRMT5 assay buffer | 2.5 µl | – | – | – |
| PRMT5/MEP50 (20-40 ng/µl) | – | 2.5 µl | 2.5 µl | 2.5 µl |
| **Total** | **10 µl** | **10 µl** | **10 µl** | **10 µl** |

8) Thaw **PRMT5 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT5 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **PRMT5 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

9) Dilute **PRMT5 enzyme** in **1× PRMT5 assay buffer** at 20-40 ng/µl (50-100 ng/2.5 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

10) Initiate reaction by adding 2.5 µl of diluted **PRMT5** prepared as described above to the wells labeled "Positive Control", "Test Inhibitor", and "Substrate Control". Incubate at room temperature for two hours.**Error! No bookmark name given.** Cover the plate with a plate sealer if necessary.

**Step 2:**

1) Thaw **TR-FRET Detection Buffer** on ice.
2) Dilute **Eu-labeled antibody** 1000-fold with **TR-FRET Detection Buffer**.
3) Add 5 µl per well. Incubate 30 minutes at room temperature with slow shaking.

**Step 3**:

1) Dilute **Dye-labeled acceptor** 100-fold with **TR-FRET Detection Buffer**.
2) Add 5 µl per well. Incubate for 30 min. at room temperature with slow shaking.

   *(Alternatively, dilute Eu-labeled antibody (1:2000) and Dye-labeled acceptor (1:200) with TR-FRET Detection Buffer in one step. Add 10 µl of Antibody/Acceptor mixture per well and incubate 1 hour.)*

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3) Read the fluorescent intensity in a microtiter-plate reader capable of measuring TR-FRET.

**Instrument Settings**

| | |
|---|---|
| Reading Mode | Time Resolved |
| Excitation Wavelength | 317±20 nm |
| Emission Wavelength | 620±10 nm |
| Lag Time | 60 µs |
| Integration Time | 500 µs |
| Excitation Wavelength | 317±20 nm |
| Emission Wavelength | 665±10 nm |
| Lag Time | 60 µs |
| Integration Time | 500 µs |

**CALCULATING RESULTS:**

Two sequential measurements should be conducted. Eu-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

When percentage activity is calculated, the FRET value from the negative control (Blank or Substrate Control) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\% \text{ Activity} = \frac{FRET_s - FRET_{neg}}{FRET_p - FRET_{neg}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{neg}$ = negative control FRET, and $FRET_P$ = Positive control FRET.

**Example of Assay Results:**

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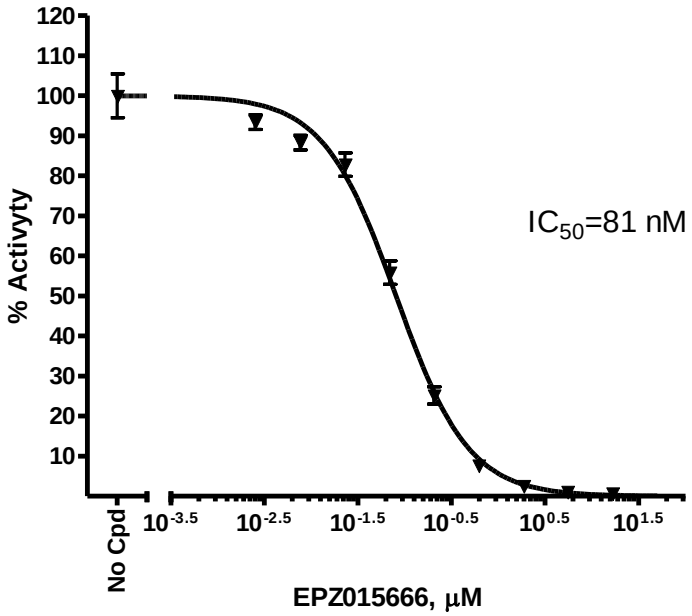
**PRMT5 Activity**




PRMT5 enzyme activity, measured using the *PRMT5 TR-FRET Assay Kit*, BPS Bioscience # …. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

**RELATED PRODUCTS**

| Product Name | Catalog # | Size |
|---|---|---|
| PRMT1 Homogeneous Assay Kit | #52054 | 384 reactions |
| PRMT3 Homogeneous Assay Kit | #52055 | 384 reactions |
| PRMT6 Homogeneous Assay Kit | #52056 | 384 reactions |
| PRMT8 Homogeneous Assay Kit | #52058 | 384 reactions |
| PRMT1 Chemiluminescent Assay Kit | #52004L | 96 reactions |
| PRMT3 Chemiluminescent Assay Kit | #52005L | 96 reactions |
| PRMT4 Chemiluminescent Assay Kit | #52041L | 96 reactions |
| PRMT5 Chemiluminescent Assay Kit | #52002L | 96 reactions |
| PRMT6 Chemiluminescent Assay Kit | #52046 | 96 reactions |
| PRMT5 recombinant protein (HEK293) | #51045 | 20 µg |
| MTAP, GST-tag | #50305 | 50 µg |
| PRMT5/MEP50 recombinant protein (Sf9) | #51048 | 20 µg |

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| PRMT1 recombinant protein (*E. coli*) | #51040 | 50 µg |
| PRMT1 recombinant protein (Sf9) | #51041 | 20 µg |
| PRMT3 recombinant protein | #51043 | 50 µg |
| PRMT4 (CARM 1) recombinant protein | #51047 | 20 µg |
| PRMT6 recombinant protein | #51049 | 20 µg |
| PRMT7 recombinant protein | #51054 | 20 µg |
| PRMT8 recombinant protein | #51052 | 20 µg |
| PRMT9 recombinant protein | #51053 | 20 µg |