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Data Sheet
L3MBTL1 TR-FRET Assay Kit
Catalog # 55200

DESCRIPTION: The L3MBTL1 TR-FRET Assay Kit is designed to measure the inhibition of L3MBTL1 binding to its substrate in a homogeneous 384 reaction format. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay procedure is straightforward and simple; a sample containing terbium-labeled donor, dye-labeled acceptor, L3MBTL1, substrate, and an inhibitor is incubated for sixty minutes. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
55000	GST-L3MBTL1(191-530)	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	Methylated MBT Ligand 1	50 µl	-80°C	
	Non-Methylated MBT Ligand 1	15 µl	-20°C	
	Tb-labeled donor	20 µl	-20°C	
	Dye-labeled acceptor	20 µl	-20°C	
	3x L3MBTL1 TR-FRET Assay Buffer	4 ml	-20°C	
Fisher 07-200-330	Nonbinding Corning, low volume, microtiter plate	1 plate	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
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(S): Min, J., *et al. Nat Struct Mol Biol* 2007; Dec;**14(12):**1229-30.

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

Protocol for L3MBTL1 assay

1) Dilute one part **3x L3MBTL1 TR-FRET Assay Buffer** with 2 parts distilled water (3-fold dilution) to make **1x L3MBTL1 Assay Buffer**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

2) Dilute **Tb-labeled donor** and **Dye-labeled acceptor** 100-fold in **1x L3MBTL1 Assay Buffer**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.

3) Add 5 µl of diluted **Tb-labeled donor**, and 5 µl of diluted **Dye-labeled acceptor** to each well designated "Test Inhibitor", "Substrate Control", and "Positive Control".

4) Add 2 µl of inhibitor solution to each well designated "Test Inhibitor". Add 2 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled "Substrate Control", and "Positive Control".

	Substrate Control	Positive Control	Test Inhibitor
Tb-labeled donor	5 µl	5 µl	5 µl
Dye-labeled acceptor	5 µl	5 µl	5 µl
Test Inhibitor	–	–	2 µl
Inhibitor Buffer (no inhibitor)	2 µl	2 µl	–
Methylated MBT Ligand 1		5 µl	5 µl
Non-Methylated MBT Ligand 1	5 µl	–	–
L3MBTL1 3.5 ng/µl	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

5) Thaw **Methylated MBT Ligand 1** and **Non-Methylated MBT Ligand 1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot each ligand into single-use aliquots. Store remaining undiluted ligand at –80°C immediately. *Note: each ligand is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.*

6) Individually dilute each ligand 40-fold in **1x L3MBTL1 Assay Buffer**. Add 5 µL of diluted **Methylated MBT Ligand 1** to each well designated as "Positive Control" and "Test Inhibitor". Add 5 µL of diluted **Non-Methylated MBT Ligand 1** to the wells labeled as "Substrate Control".

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7) Thaw **L3MBTL1** protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **L3MBTL1** protein into single-use aliquots. Store remaining undiluted **L3MBTL1** in aliquots at –80°C immediately. *Note: L3MBTL1 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

8) Dilute **L3MBTL1** in **1x L3MBTL1 Assay Buffer** to 3.5 ng/µl (10.5 ng/reaction). Initiate reaction by adding 3 µl of diluted **L3MBTL1** to wells designated for the "Substrate Control" "Positive Control", and "Test Inhibitor". Discard any remaining diluted L3MBTL1 protein after use.

9) Incubate at room temperature for 1 hour.

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

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	340±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS:

Two sequential measurements should be conducted. Tb-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 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.

$$\%\,Activity = \frac{FRET_S - FRET_{Sub}}{FRET_P - FRET_{Sub}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{Sub}$ = Substrate control FRET, and $FRET_P$ = Positive control FRET.

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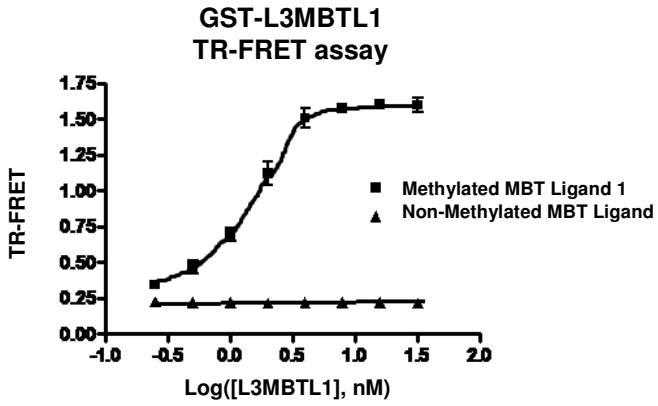
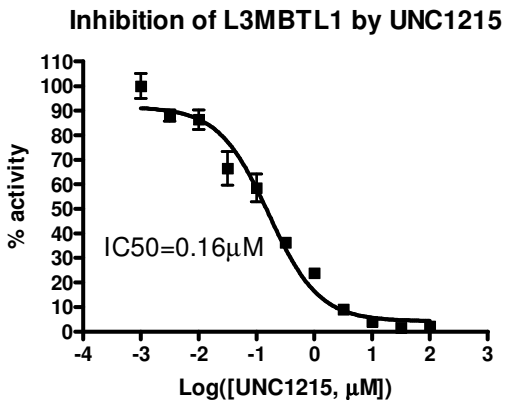
EXAMPLE OF ASSAY RESULTS:





Interaction of L3MBTL1 (BPS Bioscience #55000) with peptide ligands, assayed using the L3MBTL1 TR-FRET Assay Kit (#55200). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
L3MBTL1, GST-tag	55000	100 µg
L3MBTL1, His-tag	55002	100 µg
L3MBTL1 Inhibitor Screening Kit	55100	384 rxns.
L3MBTL2, His-tag	55018	100 µg
L3MBTL3, GST-tag	55013	100 µg
L3MBTL3, His-tag	55014	100 µg
UHRF1 (2-793), His-FLAG tag	55001	50 µg
UHRF1 (108-286), His-tag	55004	100 µg
UHRF1 (108-286), GST-tag	55003	100 µg
CBX1, GST-tag	55009	100 µg
CBX2, GST-tag	55011	100 µg
CBX7, His-tag	55017	100 µg
CHD2, GST-tag	55005	100 µg
CDY1, GST-tag	55007	100 µg
UNC1215	27404	1 mg
UNC1215	27405	5 mg
UNC1215	27406	10 mg