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Data Sheet

L3MBTL1 Inhibitor Screening Assay Kit

Catalog # 55100

DESCRIPTION: The *L3MBTL1 Inhibitor Screening Assay Kit* is designed to measure the inhibition of L3MBTL1 binding to its substrate. The kit comes in a convenient AlphaLISA® format, with enough biotinylated histone peptide substrate, assay buffer, detection buffer and purified GST-tagged L3MBTL1 MBT domain to perform a total of 384 enzyme reactions. The key to the kit is the specific binding of the L3MBTL1 MBT domain to the methylated-peptide substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing L3MBTL1 and an inhibitor of choice is incubated with the biotinylated substrate for thirty minutes. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
55000	GST- L3MBTL1 (191-530)	80 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	Methylated MBT Ligand 1	400 µl	-80°C	
	Non-Methylated MBT Ligand 1	200 µl	-80°C	
	3x L3MBTL1 assay buffer	4 ml	-20°C	
	3x L3MBTL1 detection buffer	3 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Glutathione AlphaLISA® Acceptor Beads, 5 mg/ml (PerkinElmer #AL109C)
AlphaScreen® Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)
Optiplate-384 (PerkinElmer #6007290)
AlphaScreen® microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Useful for the study of MBT domain binding assays, screening inhibitors, and selectivity profiling.

CONTRAINDICATIONS: Green and blue dyes that absorb light in the AlphaScreen® signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen® assays.

STABILITY: At least one year from date of receipt when stored as directed.

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REFERENCE: Min, J., *et al., Nat Struct Mol Biol.* 2008 Jan;**15(1):**114.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

Step 1:

1) Prepare the master mixture: N wells × (2.5 µl **3× L3MBTL1 assay buffer** + 1 µl **Methylated MBT Ligand 1** + 1.5 µl **H_2O**).

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

3) Dilute **L3MBTL1** in 1x **L3MBTL1 assay buffer** to 80 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

   Add 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 **3× L3MBTL1 assay buffer** + 1.5 µl **H_2O** +1 µl of **Non-methylated Ligand 1.**

	Blank	Substrate Control	Positive Control	Test Inhibitor
3x L3MBTL1 assay buffer	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Methylated MBT Ligand 1	1 µl	–	1 µl	1 µl
Non-Methylated MBT Ligand 1	-	1 µl	-	-
H_2O	1.5 µl	1.5 µl	1.5 µl	1.5 µl
Test Inhibitor/Activator	–	–	–	2.5 µl
Inhibitor buffer (no inhibitor)	2.5 µl	2.5 µl	2.5 µl	–
1x L3MBTL1 assay buffer	2.5 µl	–	–	–
L3MBTL1 (80 ng/µl)	–	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

4) Add 2.5 µl of **inhibitor solution** to each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 2.5 µl of the same **solution without inhibitor** (inhibitor buffer). *Note: Keep DMSO concentration below 0.5 %.*

5) Add 2.5 µl of **1x L3MBTL1 assay buffer** to the well designated "Blank".

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6) Initiate reaction by adding 2.5 µl of diluted L3MBTL1 prepared as described above to the wells labeled "Positive Control", "Test Inhibitor", and "Substrate Control". Incubate at room temperature for 30 minutes.

Step 2:

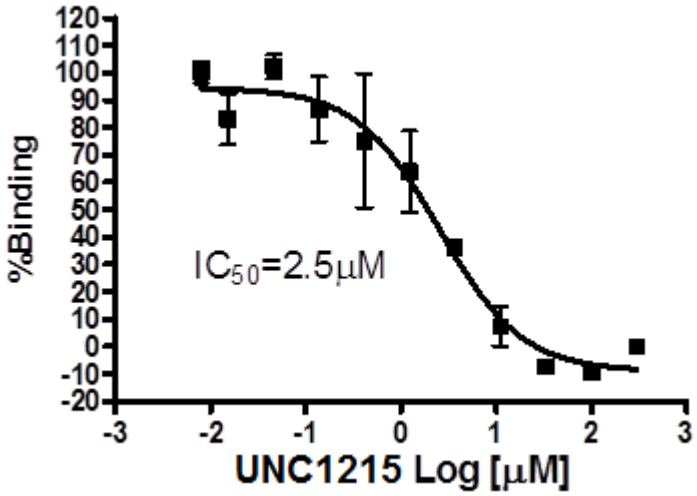
Note: Protect your samples from direct exposure to light!

1) Dilute Glutathione AlphaLISA® Acceptor Beads (PerkinElmer #AL109C) 250-fold with 1x L3MBTL1 detection buffer. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with 1x L3MBTL1 detection buffer. Add 10 µl per well. Incubate at room temperature for 15 - 30 minutes.
2) Read Alpha-counts.

EXAMPLE OF ASSAY RESULTS:



Inhibition of L3MBTL1 binding, measured using the L3MBTL1 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #55100. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
L3MBTL1, GST-tag	55000	100 µg
L3MBTL1, His-tag	55002	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
CHD2, GST-tag	55005	100 µg
CDY1, GST-tag	55007	100 µg