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Data Sheet

CBX1 Inhibitor Screening Assay Kit

Catalog # 79052

DESCRIPTION: The *CBX1 Inhibitor Screening Assay Kit* is designed to measure the inhibition of CBX1 binding to its substrate. The kit comes in a convenient AlphaLISA® format, with enough biotinylated peptide substrate, assay buffer, detection buffer and purified GST-tagged CBX1 chromodomain to perform a total of 384 enzyme reactions. The key to the kit is the specific binding of the CBX1 chromodomain to the methylated-peptide substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing CBX1 and an inhibitor of choice is incubated with the biotinylated substrate for one hour. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
55009	CBX1, GST-tag (2-185)	1 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	Methylated Ligand 1	400 µl	-80°C	
	Non-Methylated Ligand 1	200 µl	-80°C	
	3x CBX1 Assay Buffer	4 ml	-20°C	
	3x CBX1 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 chromodomain 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 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: Kaustov, L., *et al. J. Biol. Chem.* 2011; **286**(1):521-9.

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× CBX1 Assay Buffer** + 1 µl **Methylated Ligand 1** + 1.5 µl distilled **H_2O**). 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× CBX1 Assay Buffer** + 1.5 µl distilled **H_2O** +1 µl of **Non-methylated Ligand 1.**

2) Prepare **1× CBX1 Assay Buffer** by diluting **3× CBX1 Assay Buffer** with water. Dilute only enough **3× CBX1 Assay Buffer** as required for the assay. Store remaining **3× CBX1 Assay Buffer** at -20°C in single-use aliquots.

3) 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 1%.*

	Blank	Substrate Control	Positive Control	Test Inhibitor
3x CBX1 assay buffer	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Methylated Ligand 1	1 µl	–	1 µl	1 µl
Non-Methylated Ligand 2	–	1 µl	–	–
H_2O	1.5 µl	1.5 µl	1.5 µl	1.5 µl
Test Inhibitor	–	–	–	2.5 µl
Inhibitor buffer (no inhibitor)	2.5 µl	2.5 µl	2.5 µl	–
1x CBX1 assay buffer	2.5 µl	–	–	–
CBX1 (0.8-1 ng/µl)	–	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

4) Thaw **CBX1** 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

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undiluted protein in aliquots at -80°C immediately. *Note: **CBX1** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

5) Dilute **CBX1** in 1**× CBX1 Assay Buffer** to 0.8-1 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

6) Add 2.5 µl of **1× CBX1 Assay Buffer** to the well designated "Blank".

7) Initiate reaction by adding 2.5 µl of **diluted CBX1** prepared as described above to the wells labeled "Positive Control", "Test Inhibitor", and "Substrate Control". Incubate at room temperature for 60 minutes.

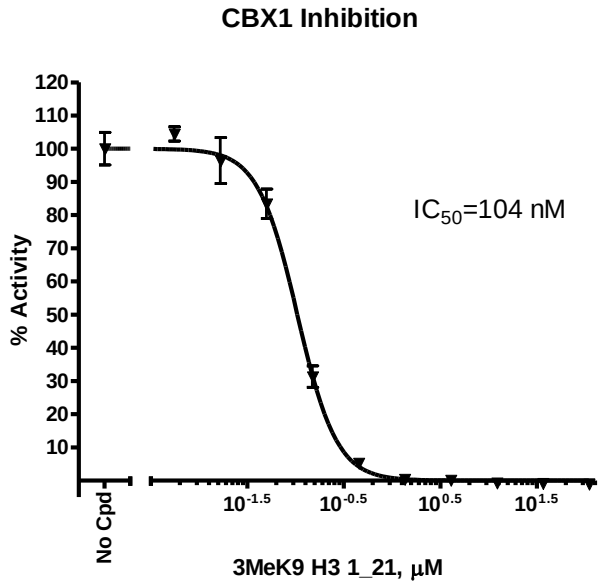
Step 2:

Note: Protect your samples from direct exposure to light!

1) Dilute Glutathione AlphaLISA Acceptor Beads (PerkinElmer #AL109C) 250-fold with **1× CBX1 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 **1× CBX1 Detection Buffer.** Add 10 µl per well. Incubate at room temperature for 5 - 10 minutes.
2) Read Alpha-counts.



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Inhibition of CBX1 binding, measured using the CBX1 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #79052. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

RELATED PRODUCTS:

Product Name	Catalog #	Size
CBX1, GST-tag	55009	100 µg
CBX2, GST-tag	55011	100 µg
CHD2, GST-tag	55005	100 µg
CDY1, GST-tag	55007	100 µg
L3MBTL1, GST-tag	55000	100 µg
L3MBTL1, His-tag	55002	100 µg
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
L3MBTL1 TR-FRET Assay Kit	55200	384 rxns.
L3MBTL1 Inhibitor Screening Assay Kit	55100	384 rxns.