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Data Sheet

SMARCA2 Inhibitor Screening Assay Kit

Catalog # 32610
Size: 384 reactions

DESCRIPTION: The *SMARCA2 Inhibitor Screening Assay Kit* is designed to measure the inhibition of SMARCA2 binding to its substrate. The *SMARCA2 Inhibitor Screening Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone peptide substrate, assay buffer, detection buffer and purified SMARCA2 bromodomain to perform a total of 384 enzyme reactions. The key to the *SMARCA2 Inhibitor Screening Assay Kit* is the specific binding of the SMARCA2 bromodomain to the acetylated histone substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing SMARCA2 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	
31141	GST-SMARCA2 (1375-1511)	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	Bromodomain Ligand 2	500 µl	-80°C	
	Non-acetylated Ligand 2	500 µl	-80°C	
33007	3x BRD Homogeneous Assay Buffer 2	4 ml	-20°C	
33006	3x BRD Homogeneous Detection Buffer 2	3 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA® GSH acceptor beads, 5 mg/ml (PerkinElmer #AL109C)
AlphaScreen® Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)
Optiplate -384 (PerkinElmer #6007290)
AlphaScreen® microplate reader

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

CONTRAINDICATIONS: DMSO above 0.5%. Only limited amounts of DMSO can be included, as it has been shown to disrupt BRD-ligand interaction. Avoid 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: Filippakopoulos, P., *et al., Cell* 2012; **149**:214.

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 **3x BRD Homogeneous Assay Buffer 2** + 1 µl **Bromodomain Ligand 2** + 1.5 µl **H_2O**).

2) 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 **3x BRD Homogeneous Assay Buffer 2** + 1.5 µl **H_2O** +1 µl of **Non-acetylated Ligand 2.**

3) Thaw **SMARCA2** 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: **SMARCA2** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

4) Dilute **SMARCA2** in **1x BRD Homogeneous Assay Buffer 2** at 2 – 4 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Substrate Control	Positive Control	Test Inhibitor
3x BRD Homogeneous Assay Buffer 2	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Bromodomain Ligand 2	1 µl	–	1 µl	1 µl
Non-acetylated Ligand 2	-	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 BRD Homogeneous Assay Buffer 2	2.5 µl			
SMARCA2 (2-4 ng/µl)	–	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

5) 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%.*

6) Add 2.5 µl of **1x BRD Homogeneous Assay Buffer 2** to the well designated "Blank".

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7) Initiate reaction by adding 2.5 µl of diluted **SMARCA2** prepared as described above to the wells labeled "Positive Control", "Substrate Control", and "Test Inhibitor". Incubate at room temperature for 30 minutes.

Step 2:

Note: Protect your samples from direct exposure to light!

1) Dilute GSH acceptor beads (PerkinElmer #AL109C) 250-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

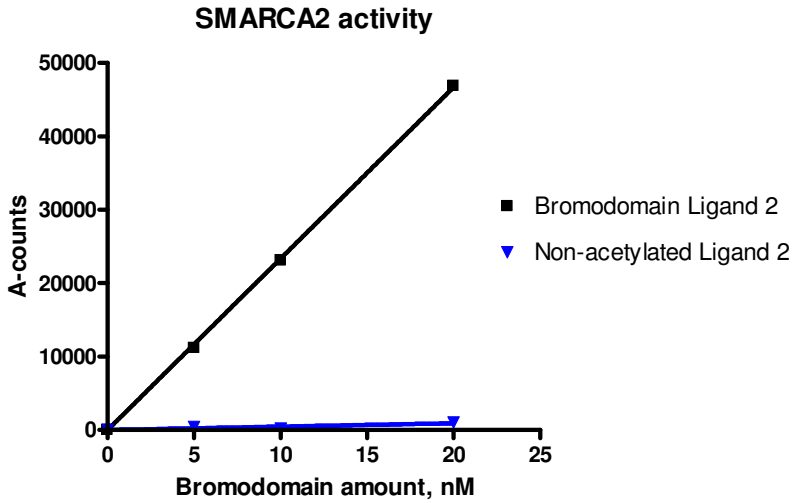
Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002) 125-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 µl per well. Incubate at room temperature for 10-15 minutes.

2) Read Alpha-counts.

Due to lot to lot variability in AlphaScreen® *bead performance, it may be necessary to optimize assay conditions. For example, slight adjustments to bromodomain or ligand concentrations may improve signal-to-noise ratio.*

Example of Assay Results:



SMARCA2 binding activity, measured using the SMARCA2 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #32610. *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
SMARCA2 (1375 – 1511), GST-tag	31141	100 µg
SMARCA2 (1375 – 1511), His-tag	31111	100 µg
BRG1 (SMARCA4) (1480-1603), GST-tag	31132	100 µg
BRG1 (SMARCA4) (1480-1603), His-tag	31102	100 µg
PB1 (BD4), His-tag	31122	100 µg
PB1 (BD6), His-tag	31133	100 µg
WDR9 (1308 – 1436), His-tag	31115	100 µg
Bromodomain Ligand 2	33003	0.5 mL
Bromodomain Nonacetylated Ligand 2	33004	0.5 mL