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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

CECR2 Inhibitor Screening Assay Kit

Catalog # 32611
Size: 384 reactions

DESCRIPTION: The CECR2 Inhibitor Screening Assay Kit is designed to measure the inhibition of CECR2 binding to its substrate. The CECR2 Inhibitor Screening Assay Kit comes in a convenient AlphaLISA® format, with biotinylated histone peptide substrate, assay buffer, detection buffer and purified CECR2 (Cat Eye Syndrome Chromosome Region 2) bromodomain to perform a total of 384 enzyme reactions. The key to the CECR2 Inhibitor Screening Assay Kit is the highly specific binding of the CECR2 bromodomain to the acetylated histone substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing CECR2 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	
31046	His-CECR2 (430-543)	100 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	BET Bromodomain Ligand	400 µl	-80°C	
	Non-acetylated Ligand 1	200 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:
Nickel Chelate AlphaLISA® Acceptor Beads, 5 mg/ml (PerkinElmer #AL108C)
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 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.

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

REFERENCE: Lee S-K, *et al., Mol. Cells* 2012, **34(1):**85-91.

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 **BET Bromodomain Ligand** + 1.5 µl **H_2O**).

2) Thaw **CECR2** on ice. Upon first thaw, briefly spin tube containing protein to recover full content of the tube. Aliquot both proteins into single use aliquots. Store remaining undiluted protein in aliquots at -80°C immediately. *Note: **CECR2** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

3) Dilute **CECR2** in **1x BRD Homogeneous Assay Buffer 2** at 100 ng/µl. Keep diluted proteins on ice until use. Discard any unused diluted protein after use.

4) 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 µl **Non-acetylated Ligand 1** + 1.5 µl H_2O.

	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
BET Bromodomain Ligand	1 µl	–	1 µl	1 µl
Non-acetylated 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 BRD Homogeneous Assay Buffer 2	2.5 µl			
CECR2 (100 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).

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 **CECR2** prepared as described above to each well 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 Nickel Chelate AlphaLISA® Acceptor Beads (PerkinElmer #AL108C) 250-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

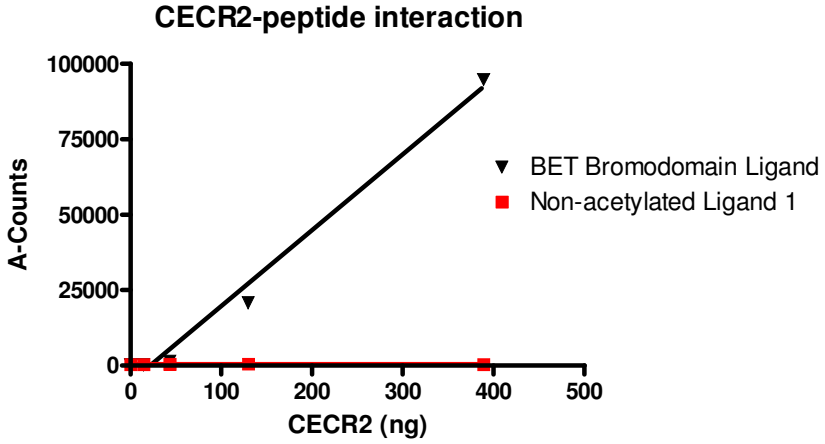
Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 µl per well. Incubate at room temperature for 15 – 30 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:



CECR2 binding activity, measured using the CECR2 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #32611. *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
CECR2 (430-543), His-tag*	31046	100 µg
FALZ/BPTF (2791-2911), His-tag*	31131	100 µg
GCN5 (727 – 837), His-tag	31114	100 µg
PCAF (720 – 832), His-tag	31120	100 µg
TAF1 (1400 – 1518), His-tag	31123	100 µg
TAF1 (1519 – 1657), His-tag*	31110	100 µg
TAF1L (1400 – 1651), GST-tag	31124	100 µg
TAF1L (1398 – 1516), His-tag*	31103	100 µg
TAF1L (1517 – 1649), His-tag*	31104	100 µg
BET Bromodomain Ligand	33000	0.5 mL
Non-acetylated Ligand 1	33005	0.5 mL
BRD4 (BD1+BD2) Inhibitor Screening Kit	32504	384 rxns.
TAF1 (BD2) Inhibitor Screening Kit	32624	384 rxns.
TAF1 (BD1+BD2) Inhibitor Screening Kit	32604	384 rxns.
TAF1L (BD2) Inhibitor Screening Kit	32602	384 rxns.
TAF1L (BD1+BD2) Inhibitor Screening Kit	32603	384 rxns.
(+)-JQ1 Inhibitor	27401	1 mg

**Also available with GST-tag*