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Data Sheet

FBXL10 Homogeneous Assay Kit

Catalog # 50610

DESCRIPTION:

The *FBXL10 Homogeneous Assay Kit* is designed to measure the activity of the FBXL10 for screening and profiling applications. FBXL10, also known as KDM2B, JHDM1B, and CXXC2, is a histone lysine demethylase that exhibits demethylation activity toward trimethyl-lysine 4 (H3K4me3) and dimethyl-lysine 36 (H3K36me2) on histone H3. The *FBXL10 Homogeneous Assay Kit* comes in a convenient AlphaLISA[®] format, with biotinylated histone H3 peptide substrate, primary antibody, demethylase assay buffer, and purified FBXL10 for 384 enzyme reactions. The key to the *FBXL10 Homogeneous Assay Kit* is a highly specific antibody that recognizes demethylated substrate. With this kit, only three simple steps on a microtiter plate are required for demethylase activity detection. First, a sample containing FBXL10 enzyme is incubated with the biotinylated substrate. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50120	FBXL10	15 µg	-80 °C	Avoid Freeze/ Thaw Cycles
52140P	Primary antibody 16	10 µl	-80 °C	
	Biotinylated histone H3 peptide substrate	10 µl	-80 °C	
	4X FBXL10 assay buffer 1	2 ml	-20 °C	
	4X FBXL10 assay buffer 2 (Incomplete Buffer)	1 ml	-20 °C	
	4X Detection buffer	2 ml	-20 °C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

AlphaLISA anti-rabbit IgG acceptor beads, 5 mg/ml (PerkinElmer #AL104L)
AlphaScreen Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)
Optiplate 384 (PerkinElmer #6007290)
AlphaScreen microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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 (NaN₃) or metal ions (Fe²⁺, Fe³⁺, Cu²⁺, Zn²⁺ and Ni²⁺). The presence of culture medium RPMI 1640 at >1% leads to 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: Iwase, S., *et al. Cell* 2007; **128**(6):1077-1088.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

- 1) Dilute **Biotinylated histone H3 peptide substrate** 40-fold with water. Dilute only the amount required for the assay. Discard any unused **Diluted Biotinylated histone H3 peptide substrate** after use.
- 2) Prepare master mix: N wells × (2.5 µl **4X FBXL10 assay buffer 1** + 1 µl **Diluted Biotinylated histone H3 peptide substrate** + 0.5 µl water).
- 3) Add 4 µl of master mix to each well designated for the "Positive Control" and "Test Inhibitor". For the wells labeled "Blank", add 2.5 µl **4X FBXL10 assay buffer 2 (Incomplete buffer)** + 1 µl **Diluted Biotinylated histone H3 peptide substrate** + 0.5 µl water. Note: The incomplete buffer, which does not contain α-ketoglutarate, provides a more accurate background value than a no-enzyme control.
- 4) Add 3 µl of inhibitor solution to each well designated "Test Inhibitor". For the wells designated "Positive Control" and "Blank" add 3 µl of the same solution without inhibitor (Inhibitor buffer).
- 5) Thaw **FBXL10** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **FBXL10** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **FBXL10** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

Reagent	Blank	Positive Control	Test Inhibitor
4X FBXL10 assay buffer 1	—	2.5 µl	2.5 µl
4X FBXL10 assay Buffer 2 (Incomplete buffer)	2.5 µl	—	—
Biotinylated Substrate (Diluted)	1 µl	1 µl	1 µl
Distilled water	0.5 µl	0.5 µl	0.5 µl
Test Inhibitor/Activator	—	—	3 µl
Inhibitor buffer (no inhibitor)	3 µl	3 µl	—
FBXL10 (10 ng/µl)	3 µl	3 µl	3 µl
Total	10 µl	10 µl	10 µl

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- 6) Dilute **FBXL10** in **1X FBXL10 assay buffer 2 (Incomplete Buffer)** at 10 ng/ μ l (30 ng/3 μ l). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
- 7) Initiate reaction by adding 3 μ l of diluted **FBXL10** prepared as described above to all wells. Incubate at room temperature for one hour. *Note: All incubations should be performed with slow shaking on a rotator platform.*

Note: Protect your samples from direct exposure to light for steps 2 and 3!

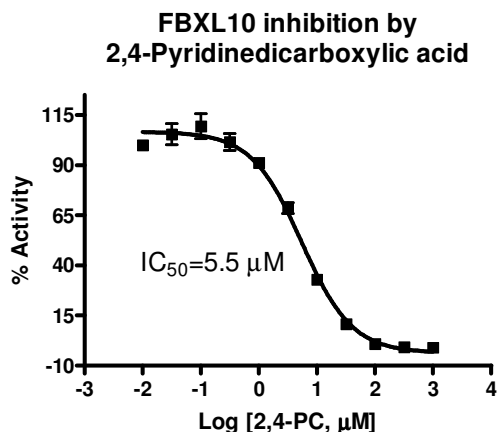
Step 2:

- 1) Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104L) 250-fold with **1X Detection buffer**. Add 5 μ l per well. Manually shake plate briefly.
- 2) Dilute "**Primary antibody 16**" 200-fold with **1X Detection buffer**. Add 5 μ l per well. Shake on a rotator platform for 30 minutes at room temperature.
(Alternatively, dilute anti-Rabbit Acceptor beads (1:500) and Primary antibody 16 (1:400) with 1x Detection buffer in one step. Add 10 μ L of acceptor beads/antibody mixture per well.)

Step 3:

- 1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1X Detection buffer**. Add 10 μ l per well. Shake on a rotator platform for 30 minutes at room temperature.
- 2) Read Alpha-counts.

Example of Assay Results:



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Figure Legend. FBXL10 enzyme activity, measured using the FBXL10 Homogeneous Assay Kit, BPS Bioscience Cat# 50610. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product	Cat. #	Size
FBXL10(KDM2B, JHDM1B)	#50120	20 µg
FBXL11(KDM2A)	#50102	20 µg
MLL1/WAR Complex	#51022	50 µg
MLL1/WARD Complex	#51021	50 µg
SetD2	#53019	50 µg
Set7/9 (Sf9)	#50019	50 µg
Set8	#50008	50 µg
FBXL11 Assay Kit	#50611	384 rxns.
MLL1 Complex Chemiluminescent Assay Kit	#53008	96 rxns.
SETD2 Chemiluminescent Assay Kit	#52060	96 rxns.

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