Description

The FBXL11 (KDM2A) Homogeneous Assay Kit is designed to measure FBXL11 (F-box and leucine-rich repeat protein 11) activity for screening and profiling applications. The FBXL11 (KDM2A) Homogeneous Assay Kit comes in a convenient 96-well AlphaLISA® format, with enough purified recombinant FBXL11 (amino acids 2-700), substrate, primary antibody, assay and detection buffer for 100 enzyme reactions.

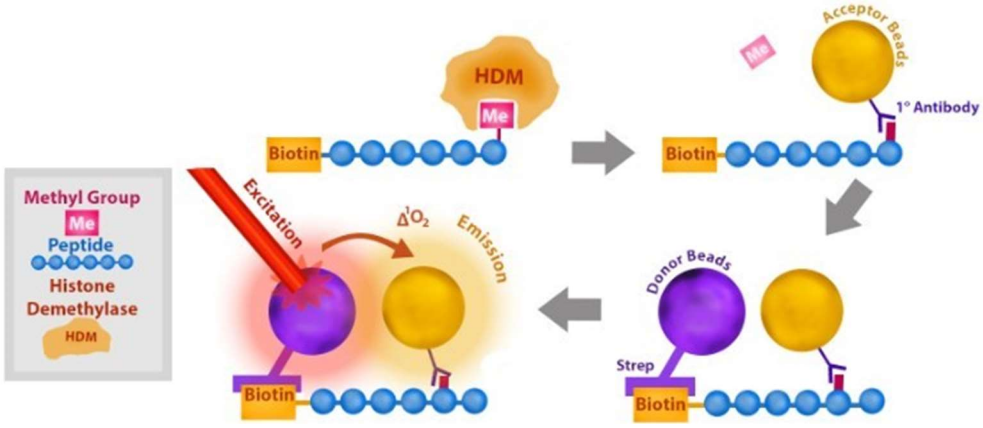

Figure 1: *FBXL11 (KDM2A) Homogenous Assay Kit schematic.*
A sample containing FBXL11 is incubated with a biotinylated substrate. This is followed by the addition of acceptor beads and primary antibody, and finally donor beads. Alpha-counts are then counted. Alpha-counts are proportional to FBXL11 demethylase activity.

Background

FBXL11 (F-box and leucine-rich repeat protein 11), also known as KMD2A (lysine demethylase 2A), belongs to the JmjC (Jumonji C domain) demethylase family of proteins. It is involved in H3 lysine 36 (H3K36) demethylation, and chromosome remodeling and gene expression, stem cell differentiation, metabolism, and DDR (DNA damage repair). It is found in the nucleus, where it can bind to cpG DNA, and it is found at high levels in the brain, testis, ovaries, and the lungs. It can also be found in many cancer types, with the notable exception of prostate cancer. FBXL11 expression is regulated by microRNA (miRNA), and its levels increase during inflammation, hypoxia or oxidative stress. For instance, in non-small cell lung cancer (NSCLC) tPA (tissue-type plasminogen activator) can activate COX2 (cyclooxygenase-2) by acting on FBXL11. FBXL11 promotes NSCLC also by binding to the DUSP3 (dual specificity phosphatase 3) promoter region, decreasing its expression, and consequently increasing phosphorylation of ERK1/2 (extracellular signal regulated kinases 1/2) and cancer metastasis. The use of FBXL11 as a therapeutic target is thus an interesting approach and is under investigation.

Application(s)

Study enzyme kinetics and screen small molecule inhibitors in high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
50156	FBXL11 (KDM2A, JHDM1A), FLAG-Avi-tags*	10 µg	-80°C
52140P-2	Primary Antibody 16-2	5 µl	-80°C
79843	Biotinylated Histone H3 Peptide Substrate	300 reactions	-80°C
79847	4x HDM Assay Buffer 5	3 ml	-80°C
52301	4x Detection Buffer	2 ml	-20°C

**The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

Name	Ordering Information
AlphaLISA® Anti-Rabbit IgG Acceptor Beads, 5 mg/ml	Perkin Elmer #AL104C
AlphaScreen® Streptavidin-Conjugated Donor Beads, 5 mg/ml	Perkin Elmer #6760002S
Optiplate - 96	Perkin Elmer #6005290
AlphaScreen® microplate reader	

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The FBXL11 (KDM2A) Homogenous Assay Kit is compatible with up to 1% final DMSO concentration.
- Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (λ=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. Media like MEM, which lacks these components, does not affect AlphaScreen assays.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test inhibitor”.
- We recommend maintaining the diluted protein on ice during use.

- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend preincubating the enzyme with inhibitor, however, it is acceptable to add the substrate mixture and inhibitor followed by diluted FBXL11 without the preincubation step.
- We recommend using Daminozide as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1:

1. Add 940 µl of distilled water to the tube containing the lyophilized Biotinylated Histone H3 Peptide Substrate.

2. Diluted 4-fold the 4x HDM Assay Buffer 5 with distilled water.

3. Prepare the Test Inhibitor (6 µl/well): for a titration prepare serial dilutions at concentrations 3.3-fold higher than the desired final concentrations. The final volume of the reaction is 20 µl.

   3.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x HDM Assay Buffer 5, 3.3-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x HDM Assay Buffer 5 (Diluent Solution).

   OR

   3.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 30-fold in 1x HDM Assay Buffer 5 to prepare the highest concentration of the 3.3-fold intermediate dilutions. The concentration of DMSO is now 3.3%.

   Prepare serial dilutions of the Test Inhibitor at 3.3-fold the desired final concentrations using 3.3% DMSO in 1x HDM Assay Buffer 5 to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 3.3% DMSO in 1x HDM Assay Buffer 5 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

4. Add 6 µl of inhibitor solution to each well designated “Test Inhibitor”.

5. Add 6 µl of Diluent Solution to the “Blank” and “Positive Control” wells.

6. Thaw FBXL11 on ice. Briefly spin the tube containing enzyme to recover the full content of the tube.

7. Dilute FBXL11 to 5 ng/µl with 1x HDM Assay Buffer 5 (6 µl/well).

8. Add 6 µl of diluted FBXL11 to the “Test Inhibitor” and “Positive Control” wells.

9. Add 6 µl of 1x HDM Assay Buffer 5 to the "Blank" wells.

10. Pre-incubate for 30 minutes at Room Temperature (RT) with slow agitation.

11. Prepare a Master Mix (8 µl/ well): N wells x (5 µl of 4x HDM Assay Buffer 5 + 3 µl of resuspended Biotinylated Histone H3 Peptide Substrate).

12. Initiate the reaction by adding 8 µl of Master Mix to all wells.

13. Incubate at RT for 1 hour with slow agitation.

Protect your samples from direct exposure to light for step 2 and 3. Photobleaching will occur!

Component	Test Inhibitor	Blank	Positive Control
Test Inhibitor	6 µl	-	-
Diluent Solution	–	6 µl	6 µl
Diluted FBXL11 (5 ng/µl)	6 µl	-	6 µl
1x HDM Assay Buffer 5	-	6 µl	-
Master Mix	8 µl	8 µl	8 µl
Total	**20 µl**	**20 µl**	**20 µl**

Step 2:

1. Dilute 4-fold the 4X Detection Buffer with distilled water. This makes 1X Detection Buffer.

   Note: Prepare only enough to perform the assay. Store remaining 4x Detection Buffer at -20°C.

2. Dilute AlphaLISA® Anti-Rabbit Acceptor Beads 500-fold and 200-fold the Primary Antibody 16-2, together in one step, in 1X Detection Buffer (10 µl of mix/well). Mix well.

3. Add 10 µl of mix to each well.

4. Incubate 30 minutes at RT.

Step 3:

1. Dilute AlphaScreen® Streptavidin-Conjugated Donor Beads 125-fold with 1x Detection Buffer (10 µl/well).

2. Add 10 µl of diluted donor beads to each well.

3. Incubate for 30 minutes at RT in a rotating platform.

4. Read Alpha-Counts.

5. The "Blank" control might be important to determine the background A-screen counts in the assay. The blank value should be subtracted from all other values.

Example Results

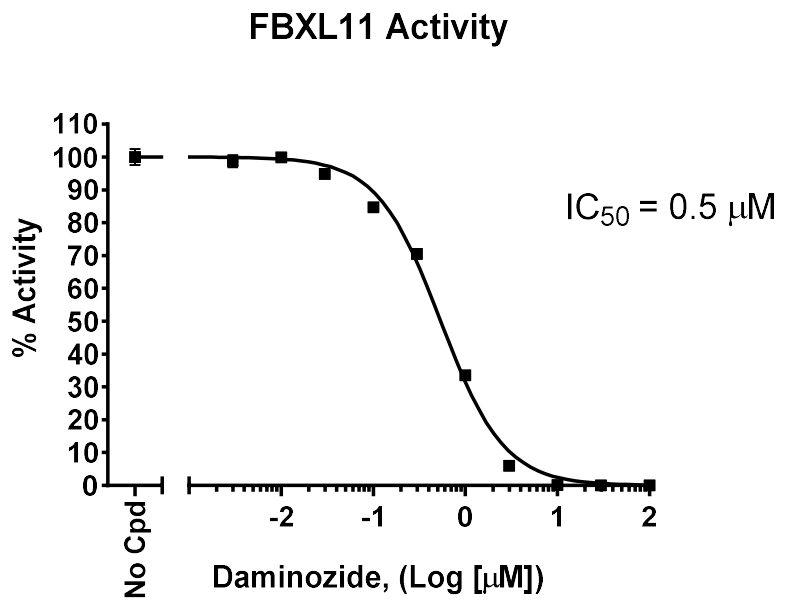

Figure 2: Inhibition of FBXL11 activity by Daminozide.
FBXL11 activity was measured in the presence of increasing concentrations of Daminozide.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Iwase S., *et al.,*2007 Cell 128(6):1077-1088.
Liu L., *et al.*, 2021 *Exp Ther Med* 22(1):723.

Related Products

Products	*Catalog #*	*Size*
FBXL10 (KDM2B) Chemiluminescence Assay Kit	50157	96 reactions
FBXL10 (KDM2B) Homogeneous Assay Kit	50610	96 reactions/384 reactions
FBXL10 (KDM2B, JHDM1B), FLAG- tag Recombinant	50120	20 µg

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