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Data Sheet

UTX Chemiluminescent Assay Kit

Catalog # 50615
Size: 96 reactions

DESCRIPTION: The *UTX Chemiluminescent Assay Kit* is designed to measure UTX activity for screening and profiling applications. UTX, also known as KDM6A, is a JmjC-domain protein that exhibits demethylation activity toward di- and trimethyl-lysine 27 on histone H3 (H3K27me2/3). The *UTX Chemiluminescent Assay Kit* comes in a convenient 96-well strip plate format, precoated with methylated histone H3 peptide substrate, primary antibody, HRP-labeled secondary antibody, demethylase assay buffer, and purified UTX for 96 enzyme reactions. The key to the *UTX Chemiluminescent 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 detection of demethylase activity. First, UTX enzyme is incubated with the methylated H3 peptide for one hour. Next, primary antibody is added. Finally, the plate is treated with an HRP-labeled secondary antibody followed by the addition of the HRP substrate to produce chemiluminescence that can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50119	UTX (KDM6A), N-term FLAG-tag	2.5 µg	-80°C	***Avoid freeze/ thaw cycles!***
52140F	Primary antibody 6	12.5 µl	-80°C	
52131H	Secondary HRP-labeled antibody 2	10 µl	-80°C	
	4x UTX direct assay buffer	3 x 1 ml	-80°C	
52100	Blocking buffer 4	50 ml	+4°C	
	HRP chemiluminescent substrate A (transparent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
	8-well strip plate module precoated with histone substrate	1	+4°C	

MATERIALS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: DMSO >1%, strong acids or bases, ionic detergents, high salt

STABILITY: One year from date of receipt when stored as directed.

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REFERENCE: Greenfield A, *et al*. 1998. *Hum. Mol. Genet.* **7(4):** 737–42.
Agger K, *et al*. 2007. *Nature* **449(7163):** 731–4.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Rehydrate the microwells by adding 200 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the strip plate onto clean paper towels to remove liquid.

2) Prepare master mix: N wells × (7.5 µl **4x UTX Direct Assay Buffer** + 12.5 µl distilled water). Add 20 µl of master mixture to each well.

3) Add 10 µl of inhibitor solution of each well designated “Test Inhibitor”. For the “Positive Control" and "Blank" add 10 µl of the same solution without inhibitor (Inhibitor buffer). *Note: Keep final DMSO concentration ≤1%.*

	Blank	Positive Control	Test Inhibitor
4x UTX direct assay buffer	7.5 µl	7.5 µl	7.5 µl
Distilled water	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor/Activator	–	–	10 µl
Inhibitor buffer (no inhibitor)	10 µl	10 µl	–
1x UTX direct assay buffer	20 µl	–	–
UTX (1.25 ng/µl)	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

4) Dilute 1 part **4x UTX Direct Assay Buffer** with 3 parts distilled water (4-fold dilution) to make **1x UTX Direct Assay Buffer**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

5) Add 20 µl of **1x UTX direct assay buffer** to wells designated as "Blank".

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

7) Dilute **UTX** in **1x UTX Direct Assay Buffer** at 1.25 ng/µl (25 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

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8) Initiate reaction by adding 20 µl of **diluted UTX** prepared as described above to wells designated "Positive Control" and "Test Inhibitor". Incubate at room temperature for one hour.

9) Wash the strip plate three times with TBST buffer. Blot dry onto clean paper towels.

10) Add 100 µl of **Blocking Buffer 4** to every well. Shake on a rotating platform for 10 minutes. Remove the supernatant from the wells.

Step 2:

1) Dilute "**Primary antibody 6"** 800-fold with **Blocking Buffer 4**.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Wash strip plate with TBST buffer and incubate in **Blocking Buffer 4** as described in steps 1-9 and 1-10.

Step 3:

1) Dilute "**Secondary HRP-labeled antibody 2**" 1,000-fold with **Blocking Buffer 4** Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

2) Wash strip plate with TBST buffer and incubate in **Blocking Buffer 4** as described in steps 1-9 and 1-10.

3) Just before use, mix on ice 50 µl **HRP chemiluminescent substrate A** and 50 µl **HRP chemiluminescent substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

4) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

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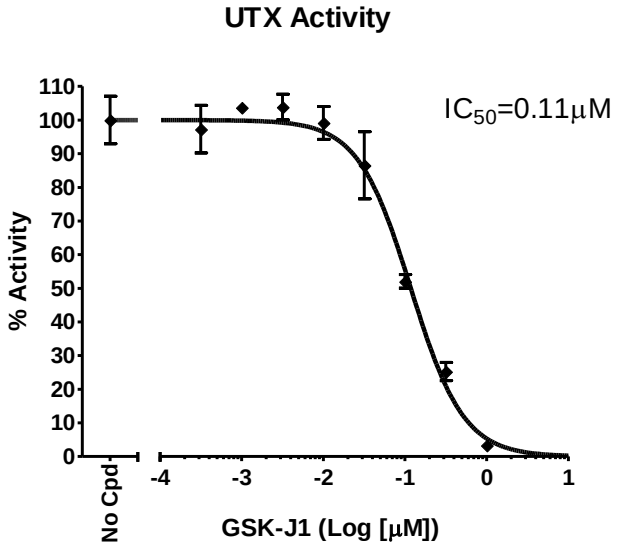
Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Examples of Assay Results:



UTX enzyme inhibition by GSK-J1, measured using the *UTX Chemiluminescent Assay Kit*, BPS Bioscience #50615. *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
UTX (KDM6A), N-terminal FLAG-tag	50119	20 µg
UTX (KDM6A), C-terminal FLAG-tag	50116	20 µg
JDJM3 (KDM6B**)**, FLAG-tag	50115	20 µg
FBXL10 recombinant protein	50120	20 µg
FBXL11 recombinant protein	50156	20 µg
UTX(KDM6A) Homogeneous Assay Kit	50516	384 reactions
JMJD3 (KDM6B) Homogeneous Assay Kit	50416	384 reactions
FBXL10 Homogeneous Assay Kit	50610	384 reactions
FBXL11 Homogeneous Assay Kit	50611	384 reactions
JARID1A Homogeneous Assay Kit	50510	384 reactions
JMJD2A Homogeneous Assay Kit	50413	384 reactions
JMJD2C Chemiluminescent Assay Kit	50405	96 reactions
JMJD3 Chemiluminescent Assay Kit	50406	96 reactions
Anti-H3K27me2 monoclonal antibody	52140F	25 µl
Anti–H3K4me3 monoclonal antibody	25256	50 µg
Anti–H3K4me3 polyclonal antibody	25257	50 µg
Anti–H3K36me2 monoclonal antibody	25247	50 µg
Anti–H3K36me2 polyclonal antibody	25248	50 µg
Anti–H3K36me3 monoclonal antibody	25249	50 µg
Anti–H3K36me3 polyclonal antibody	25250	50 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is same as “blank” value.	UTX has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh UTX, BPS Bioscience #50119. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of UTX, BPS Bioscience #50119 to create a standard curve.