6042 Cornerstone Court W, Ste B
San Diego, CA 92121
**Tel:** 1.858.202.1401
**Fax:** 1.858.481.8694
**Email:** info@bpsbioscience.com

# Data Sheet

## *FTO Chemiluminescent Assay Kit*

**Catalog # 79344**

**BACKGROUND:** The methylation of internal adenosines at the $N^6$ position ($m^6A$) in RNA has been shown to play essential roles in various normal and pathologic processes, including various cancers, leukemia, Alzheimer's disease, and obesity. FTO (Fat mass and obesity-associated protein), also known as ALKBH9 and BMIQ14, is the first $m^6A$ demethylase identified.

**DESCRIPTION:** The *FTO Chemiluminescent Assay Kit* is designed to measure FTO activity for screening and profiling applications. The *FTO Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well strip plate precoated with methylated FTO substrate, primary antibody, the secondary HRP-labeled antibody, demethylase assay buffer, and purified FTO for 96 enzyme reactions. The key to the *FTO Chemiluminescent Assay Kit* is a highly specific antibody that recognizes methylated substrate. Signal is inversely related to the activity. With this kit, only three simple steps on a microtiter plate are required for detection of demethylase activity. First, FTO enzyme is incubated with the substrate. 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 | |
|---|---|---|---|---|
| 79306 | FTO | 100 µg | -80°C | ***Avoid freeze/ thaw cycles!*** |
| 52140Z4 | Primary antibody 29 | 100 µl | -80°C | |
| 52131H | Secondary HRP-labeled antibody 2 | 10 µl | -80°C | |
| | 4x FTO assay buffer | 3 x 1 ml | -80°C | |
| 79556 | Blocking buffer 1 | 50 ml | +4°C | |
| 79670 | ELISA ECL substrate A (transparent bottle) | 6 ml | RT | |
| | ELISA ECL substrate B (brown bottle) | 6 ml | RT | |
| | White 8-well strip plate module precoated with RNA substrate* | 1 | +4°C | |

*** Make sure you are working in RNAse-free conditions.**

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**MATERIALS REQUIRED BUT NOT SUPPLIED:**
TBST buffer (1 x 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:** 6 months from date of receipt when stored as directed.

**REFERENCE:** Zhou Y., *et al. J Biomed Sci. 2017*; **24**(1):65.

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

**Step 1:**

1) Rehydrate the microwells by adding 200 µl of TBST buffer (1 x 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 FTO Assay Buffer** + 17.5 µl water). Add 25 µl of master mixture to each well.

3) Add 5 µl of inhibitor solution of each well designated “Test Inhibitor”. For the “Positive Control" and "Blank" add 5 µl of the same solution without inhibitor (Inhibitor buffer). *Note: Keep final DMSO concentration ≤1%.*

| | Blank | Positive Control | Test Inhibitor |
|---|---|---|---|
| 4x FTO assay buffer | 7.5 µl | 7.5 µl | 7.5 µl |
| Distilled water | 17.5 µl | 17.5 µl | 17.5 µl |
| Test Inhibitor/Activator | – | – | 5 µl |
| Inhibitor buffer (no inhibitor) | 5 µl | 5 µl | – |
| 1x FTO buffer | 20 µl | – | – |
| Diluted FTO (50 ng/µl) | – | 20 µl | 20 µl |
| Total | 50 µl | 50 µl | 50 µl |

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4) Prepare **1x FTO Buffer** by diluting 1 part **4x FTO Assay Buffer** with three parts distilled water. Prepare only enough **1x FTO Buffer** for the assay. Add 20 µl of **1x FTO buffer** to wells designated as "Blank".

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

6) Dilute **FTO** in **1x FTO Buffer** at 50 ng/µl (1000 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

7) Initiate reaction by adding 20 µl of **diluted FTO** prepared as described above to wells designated "Positive Control" and "Test Inhibitor". Incubate overnight at room temperature with slow shaking. Glue the wells if necessary.

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

9) Add 100 µl of **Blocking buffer** to every well. Shake on a rotating platform for 10 minutes. Remove supernatant as described above.

**Step 2:**

1) Dilute "**Primary antibody 29**" 100-fold with **Blocking Buffer 1**.

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

3) Remove supernatant from the wells and wash the strip three times with 200 µl of TBST buffer and incubate in **Blocking Buffer 1** as described in steps 1-8 and 1-9.

**Step 3:**

1) Dilute "**Secondary HRP-labeled antibody 2**" 1,000-fold with **Blocking Buffer 1**.

2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Remove supernatant from the wells and wash the strip three times with 200 µl of TBST buffer and incubate in **Blocking Buffer 1** as described in steps 1-8 and 1-9.

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

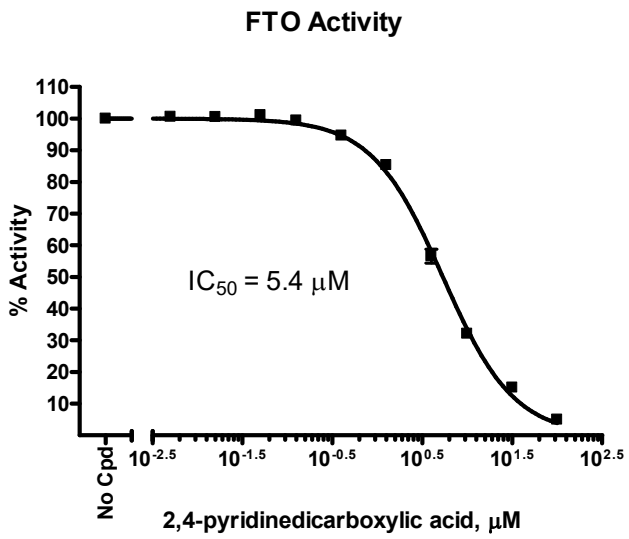
5) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

**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 40000). Make sure signal decrease corresponds to increased activity.

**Examples of Assay Results:**




FTO enzyme inhibition by 2-4-pyridinedicarboxylic acid (2,4-PC), measured using the *FTO Chemiluminescent Assay Kit*, BPS Bioscience #79344. *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 |
|---|---|---|
| JHDM1D (KDM7A) recombinant protein | 50419 | 20 µg |
| JMJD1A (KDM3A) recombinant protein | 50130 | 20 µg |
| JARID1A recombinant protein | 50155 | 20 µg |
| JARID1B recombinant protein | 50121 | 20 µg |
| JARID1C recombinant protein | 50112 | 20 µg |
| JMJD2A recombinant protein | 50123 | 100 µg |
| JMJD2B recombinant protein | 50111 | 100 µg |
| JMJD2C recombinant protein | 50105 | 100 µg |
| JMJD2E recombinant protein | 50118 | 100 µg |
| LSD1 recombinant protein | 50100 | 50 µg |
| JHDM1D (KDM7A) Homogeneous Assay Kit | 50420 | 384 reactions |
| JARID1A Homogeneous Assay Kit | 50510 | 384 reactions |
| JARID1B Homogeneous Assay Kit | 50512 | 384 reactions |
| JARID1C Homogeneous Assay Kit | 50511 | 384 reactions |
| JMJD2A Homogeneous Assay Kit | 50413 | 384 reactions |
| JMJD2B Homogeneous Assay Kit | 50414 | 384 reactions |
| JMJD2C Homogeneous Assay Kit | 50415 | 384 reactions |
| JMJD2C Chemiluminescent Assay Kit | 50405 | 96 reactions |
| JMJD2D Chemiluminescent Assay Kit | 50418 | 96 reactions |
| JMJD3 Chemiluminescent Assay Kit | 50406 | 96 reactions |
| LSD1 Chemiluminescent Assay Kit | 50109 | 96 reactions |

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**TROUBLESHOOTING GUIDE**

| Problem | Possible Cause | Solution |
|---|---|---|
| Luminescence signal of positive control reaction is same as "blank" value. | FTO has lost activity | Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh FTO, BPS Bioscience #79306. Store enzyme in single-use aliquots.<br>Increase time of enzyme incubation.<br>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.<br>Use a multichannel pipettor.<br>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.<br>Increase wash volume.<br>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%<br>Increase time of enzyme incubation. |
| | Results are outside the linear range of the assay | Use different concentrations of FTO, BPS Bioscience #79306 to create a standard curve. |