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

TET2 Chemiluminescent Assay Kit

Catalog # 50652

DESCRIPTION: The *TET2 Chemiluminescent Assay Kit* is designed to measure TET2 activity for screening and profiling applications. TET2 belongs to the Ten Eleven Translocation (TET) family proteins that catalyze 5-methylcytosine oxidation and generate 5-methylcytosine derivatives, including 5-hydroxymethylcytosine. TET2 is one of the most frequently mutated genes in myeloid malignancies. The *TET2 Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well strip plate precoated with methylated DNA substrate, primary antibody, a secondary HRP-labeled antibody, TET2 assay buffer, and purified TET2 for 100 enzyme reactions. The key to the *TET2 Chemiluminescent Assay Kit* is a highly specific antibody that recognizes hydroxymethylated substrate. With this kit, only three simple steps on a microtiter plate are required for detection of TET2 activity. First, TET2 enzyme is incubated with the methylated substrate for two hours. 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	
50162	TET2	40 µg	-80°C	***Avoid freeze/ thaw cycles!***
52140Z2	Primary antibody 27	100 µl	-80°C	
52131H	Secondary HRP-labeled antibody 2	10 µl	-80°C	
79832	4x TET2 assay buffer 1	3 ml	-80°C	
52100	Blocking buffer 4	50 ml	+4°C	
79670	ELISA ECL Substrate A (transparent bottle)	6 ml	Room Temp.	
79670	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp.	
	8-well strip plate module precoated with DNA substrate	1	+4°C	

MATERIALS OR INSTRUMENTS 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.

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

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

REFERENCE: Li Tan, Yujiang Geno Shi. *Development* 2012; **139(11):** 1895-1902.

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) Add 12.5 µl **H_2O** and 7.5 µl **4x TET2 Assay Buffer 1** to each well. Dilute **4X TET2 Assay Buffer 1** with water to make **1x TET2 Assay Buffer 1**.

3) Thaw **TET2** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **TET2** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: TET2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **TET2** in **1x TET2 Assay Buffer 1** at 20 ng/µl (400 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

5) 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 solvent). *Note: Keep final DMSO concentration ≤1%.*

	Positive Control	Test Inhibitor	Blank
H_2O	12.5 µl	12.5 µl	12.5 µl
4X TET2 Assay Buffer 1	7.5 µl	7.5 µl	7.5 µl
1X TET2 Assay Buffer 1	–	–	20 µl
Test Inhibitor	–	10 µl	–
Inhibitor solvent (no inhibitor)	10 µl	–	10 µl
TET2 (20 ng/µl)	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**

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6) Add 20 µl of **1x TET2 Assay Buffer 1** to wells designated as "Blank". Initiate reaction by adding 20 µl of **diluted TET2** prepared as described above to wells designated "Positive Control" and "Test Inhibitor". Incubate at room temperature for two hours.

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

8) Add 100 µl of Blocking buffer 4 to every well. Shake on a rotating platform for 10 min. Remove supernatant as above.

Step 2:

1) Dilute "**Primary antibody 27**" 100-fold with **Blocking buffer 4**.

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

3) Wash strip plate three times with TBST buffer and incubate in Blocking buffer 4 as in steps 1-7 and 1-8.

Step 3:

1) Dilute "**Secondary HRP-labeled antibody 2**" 1,000-fold with **Blocking buffer 4**.

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

3) Wash strip plate three times with TBST buffer and incubate in Blocking buffer 4 as in steps 1-7 and 1-8.

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) Incubate 5 minutes at room temperature, then 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.

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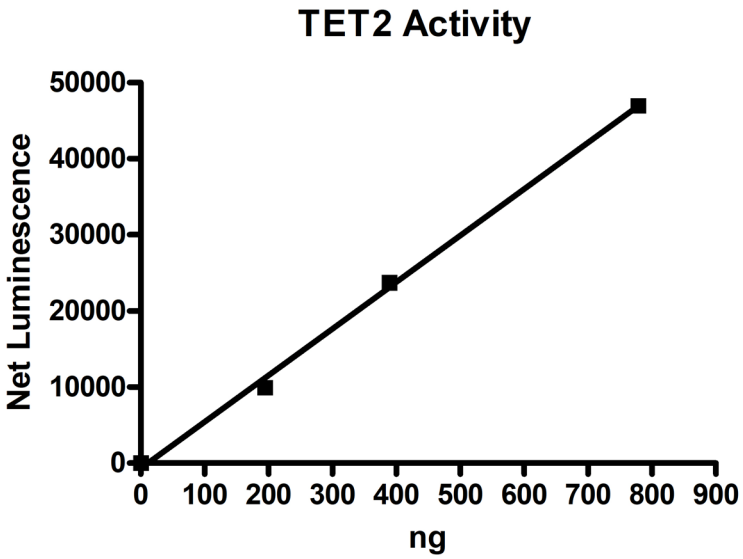
To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position 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:



TET2 enzyme activity, measured using the *TET2 Chemiluminescent Assay Kit*, BPS Bioscience #50652 *Data shown is lot-specific.*

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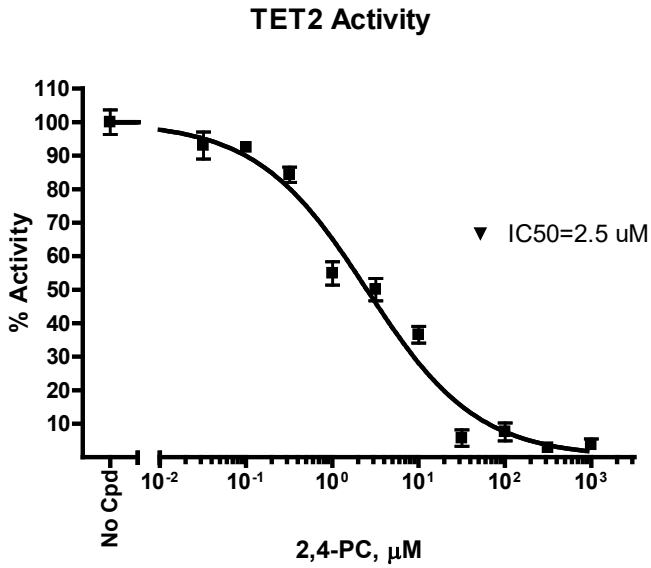

TET2 enzyme inhibition by 2,4-pyridinecarboxylic acid (2,4-PC), measured using the *TET2 Chemiluminescent Assay Kit*, BPS Bioscience #50652 *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS

Product Name	Catalog #	Size
TET1 recombinant protein	50161	50 µg
TET2 recombinant protein	50162	50 µg
TET1 Chemiluminescent Assay Kit	50651	96 reactions
Anti–5–hmC polyclonal antibody	25205	100 µl
Anti–5–mC monoclonal antibody 33D3	25207	100 µg
Anti–5–mC polyclonal antibody	25200	50 µg
MECP2 recombinant protein	50250	50 µg
DNMT1 recombinant protein	51101	10 µg
DNMT2 recombinant protein	51102	10 µg
DNMT3A/DNMT3L protein complex	51106	10 µg
DNMT3B/DNMT3L protein complex	51104	10 µg
DNMT1 Assay Kit	52050L	96 reactions
DNMT3A Assay Kit	52033	96 reactions
DNMT3B Assay Kit	52034	96 reactions

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is same as "blank" value.	TET2 has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh TET2, BPS Bioscience #50162. 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. See section on "Reading Chemiluminescence" above.
	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	Be sure to include blocking steps after wash steps. 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 TET2, BPS Bioscience #50162, to create a standard curve.