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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

TET2 Homogeneous Assay Kit

#78559
384 reactions

Description

The TET2 (Tet Methylcytosine dioxygenase 2) Homogeneous Assay Kit is designed to measure the activity of TET2 for screening and profiling applications. The TET2 Homogeneous Assay Kit comes in a convenient AlphaLISA® format, with enough biotinylated TET2 substrate, TET2 assay buffer, and purified TET2 for 384 reactions.

The key to the TET2 Homogeneous Assay Kit is a highly specific antibody that recognizes the hydroxymethylated substrate, and the increase in Alpha-counts is proportional to the hydroxymethylation of the substrate. First, a sample containing TET2 enzyme is incubated with the reaction mixture. Next, acceptor beads are added, followed by donor beads and reading the Alpha-counts.

Background

TET2 (also known as Tet Methylcytosine dioxygenase 2) belongs to the Ten Eleven Translocation (TET) family of proteins that catalyze 5-methylcytosine oxidation and generate 5-methylcytosine derivatives, including 5-hydroxymethylcytosine. TET2 regulates gene expression levels in cancer and immune cells, being a widely recognized tumor suppressor gene. Both homozygous and heterozygous mutations in TET2 are indicative of hematopoietic malignancies, with nonsense and frameshift mutations associated with poor prognosis. In addition, TET2 is one of the most frequently mutated genes in acute myeloid leukemia (AML). Targeting TET2 as therapeutical approach may be complex, as in many cancer types TET2 is inactivated, however it can still be found in some immune cell types and it can be modulated via proteins that regulate TET2 activity.

Applications

Screen for molecules that inhibit TET2 activity.

Supplied Materials

Catalog #	Name	Amount	Storage
50162	TET2, FLAG-Tag, His-Tag (catalytic domain)	3 x 40 µg	-80°C
	Biotin-labeled TET2 Substrate (4 µM)	50 µl	-80°C
	Primary Antibody 27-2	4 µl	-80°C
	4x TET2 Assay Buffer 1	3 ml	-20°C
	4x Detection Buffer 1	2 x 2 ml	-20°C
	Plate sealer	1	Room Temp.

Important: Use DNAse-free conditions!

Materials Required but Not Supplied

Name	Catalog #
AlphaLISA® anti-rabbit IgG acceptor beads	Perkin Elmer #AL104C
AlphaScreen® Streptavidin-conjugated donor beads	Perkin Elmer #6760002S
Optiplate-384	Perkin Elmer #6007290
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. **Avoid multiple freeze/thaw cycles!**

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 TET2 Homogenous Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in no higher than 4% DMSO solution in buffer and using 2.5 µl per well.
- Green and blue dyes that absorb light in the AlphaScreen® signal emission range (520-620 nm), such as Trypan Blue, interfere with the assay.
- Avoid using 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 the 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.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include a "Blank", "Positive control" and "Test inhibitor".
- We recommend preincubating antibodies or peptide-based inhibitors with TET2.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.

Step 1:

1. Prepare 1x TET2 Assay Buffer 1 by adding one part of **4x TET2 Assay Buffer 1** to three parts of distilled DNAse-free water (v/v). Mix well.

2. Thaw **TET2** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube.

3. Dilute TET2 in 1x TET2 Assay Buffer 1 to 60 ng/µl (5 µl/well). Keep the diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

   Note: If less than 384 reactions are tested, store the remaining undiluted enzyme in single-use aliquots at -80°C immediately. TET2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

4. Add 5 µl of diluted TET2 to each "Positive Control" and "Test Inhibitor" well.

5. Add 5 µl of 1x TET2 Assay Buffer 1 to the "Blank" wells.

6. Prepare the test inhibitor to be tested (2.5 µl/ well): for a titration prepare serial dilutions at concentrations 4-fold higher than the desired final concentrations. The final volume of the reaction is 10 µl.

   a. If the Test Inhibitor is water-soluble, prepare 4-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in 1x TET2 Assay Buffer 1. 1x TET2 Assay Buffer 1 is the Diluent Solution.

b. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 25-fold in 1x TET2 Assay Buffer 1 to prepare the highest concentration of the 4-fold intermediate dilutions. The concentration of DMSO is now 4%.

   For positive and negative controls, prepare 4% DMSO in 1x TET2 Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   The final concentration of DMSO should not exceed 1%.

7. Add 2.5 µl of inhibitor solution to each well designated “Test Inhibitor”.

8. For the “Positive Control" and "Blank" wells add 2.5 µl of Diluent Solution.

9. Preincubate for 30 minutes at Room Temperature (RT). Incubate with gentle agitation in a rotator platform.

10. Prepare TET2 substrate solution by diluting **Biotin-labeled TET2 Substrate** 20-fold in 1x TET2 Assay Buffer 1 (2.5 µl/well).

    Note: Calculate the amount needed and prepare only the amount required for the assay. If less than 384 reactions are tested, store the remaining undiluted enzyme in single-use aliquots at -80°C immediately.

11. Initiate the reaction by adding 2.5 µl of diluted TET2 Substrate to all wells.

12. Seal the wells with a plate sealer.

Component	Blank	Positive Control	Test Inhibitor
1x TET2 Assay Buffer 1	5 µl	-	-
Diluted TET2 (60 ng/µl)	-	5 µl	5 µl
Test Inhibitor	-	-	2.5 µl
Diluent solution	2.5 µl	2.5 µl	-
Diluted TET2 Substrate	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**

13. Incubate for two hours at RT.

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

Step 2:

1. Dilute **4x Detection buffer 1** 4-fold with DNAse-free water to make 1x Detection Buffer 1. Prepare only the amount required for the assay.

2. Dilute AlphaLISA® Anti-Rabbit acceptor beads (PerkinElmer #AL104C) 500-fold and Primary Antibody 27-2 1000-fold in the same mix in 1x Detection Buffer 1. Mix well.

3. Add 10 µl of acceptor bead/antibody mix to each reaction well. Shake on a rotator platform for 30 minutes at RT.

Step 3:

1. Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with 1x Detection Buffer 1.

2. Add 10 µl per well. Shake on a rotator platform for 15-30 minutes* at RT.

   ** The Signal-to-Noise ratio depends greatly on the performance of the beads from PerkinElmer. Duration of incubation may be extended for some lots of the beads, if necessary.*

3. Read Alpha-counts.

4. The "Blank" control is important to determine the background absorbance in the assay. The blank value should be subtracted from all other values.

Example Results

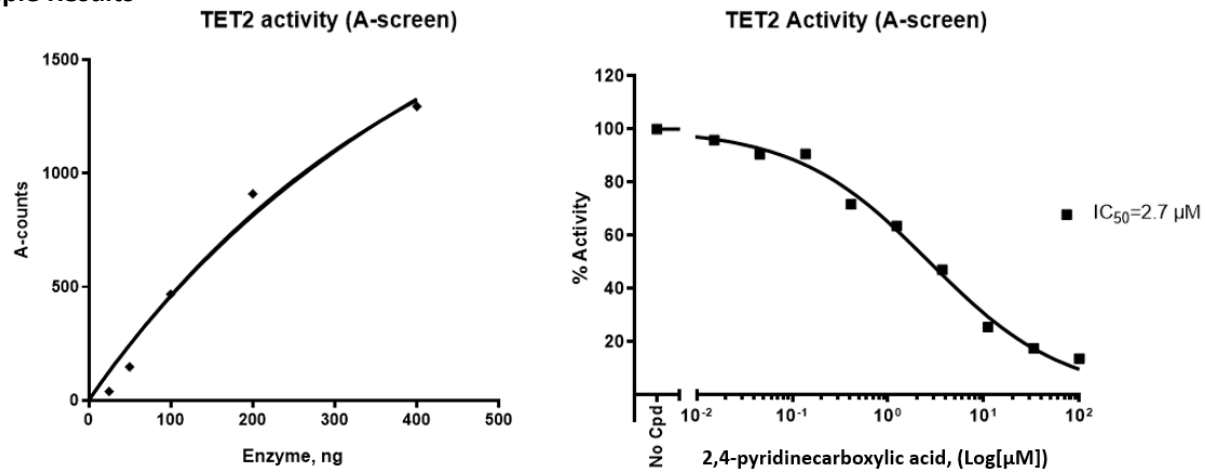

Figure 1: TET2 enzyme activity.
Left: TET2 enzyme activity was measured in the presence of increasing concentrations of TET2. Right: The inhibitory effect of 2,4-pyridinecarboxylic acid was measured in the presence of increasing concentrations of inhibitor. Results are expressed as percentage of activity relative to the positive control (measured in the absence of inhibitor and set at 100%).

Data shown is lot-specific. 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

Reference

Feng Y, *et al*., 2019 *Front Oncol* 9:210.

Related Products

Products	*Catalog #*	*Size*
TET2 Chemiluminescent Assay Kit	50652	96 reactions
TET2, FLAG-Tag, His-Tag (916-2002) Recombinant	100408	50 µg
Anti–TET2 monoclonal antibody	25320	50 µg