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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The MDM2-p53 Homogenous Assay Kit is designed to identify and assess the efficacy of inhibitors of MDM2 (mouse double minute 2 homolog) interaction with p53 by measuring their binding. The MDM2-p53 Homogeneous Assay Kit comes in a convenient 384-well AlphaLISA® format, with enough purified recombinant MDM2, p53, and buffers for 400 enzyme reactions.

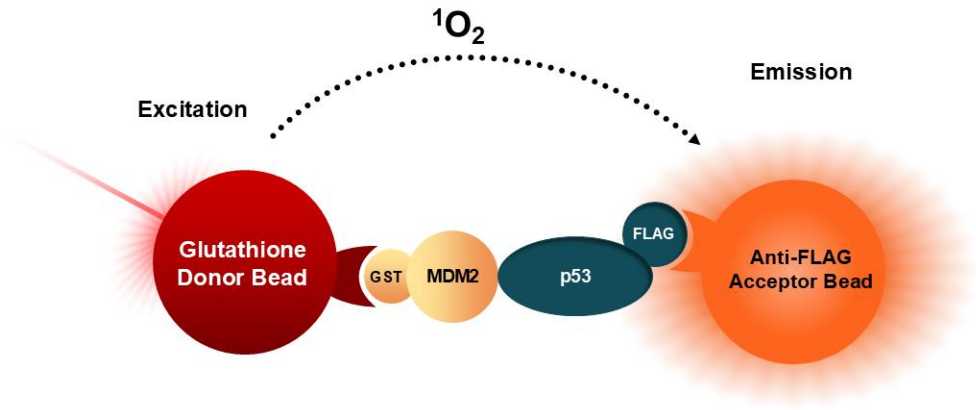

Figure 1: Illustration of the assay principle.

MDM2 contains a GST-tag, recognized by the GSH (glutathione) donor bead, while p53 contains a FLAG-tag that binds to the AlphaLISA™ acceptor bead conjugated with an anti-FLAG antibody. Upon excitation of the donor bead, a singlet oxygen is generated by the donor bead, which excites the acceptor bead and emits light proportionally to the level of MDM2-p53 interaction. AlphaLISA™ immunoassays are a no-wash alternative to ELISAs.

Background

TP53 encodes transcription factor p53, a tumor suppressor protein that maintains genomic stability and has been termed the "guardian of the genome". It is activated in response to cellular stress, such as DNA damage, oxidative stress, or oncogene activation. p53 regulates the expression of genes involved in repairing damaged DNA, preventing mutations from accumulating. It induces cell cycle arrest (commonly at the G1/S checkpoint) to allow DNA repair before cell division, and it induces apoptosis if damage is irreparable. In addition, it can regulate senescence to prevent the proliferation of damaged cells. TP53 is one of the most frequently mutated genes in human cancers, found in over 50% of cases. Mutations often result in loss of normal p53 function, although gain-of-function mutations are also observed, leading to generally more aggressive cancer phenotypes. TP53 mutation correlates with increased tumor growth, with resistance to chemotherapy, and with increased risk of metastasis. p53 protein is regulated by its interaction with MDM2 (mouse double minute 2 homolog), which serves as a ubiquitin ligase (E3) to target p53 for degradation. MDM2 ubiquitinates p53, resulting in the rapid degradation of p53 through the Ub–proteasome pathway. MDM2-mediated destabilization and inactivation of p53 plays a critical role in several human cancers. The disruption of the MDM2-p53 interaction has been regarded as an attractive strategy for anticancer drug discovery, as it would restore the tumor suppressor function of p53.

Application(s)

Study binding and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100409	MDM2, GST-tag*	2 µg	-80°C
100412	p53, FLAG-tag*	< 1 µg	-80°C
78856	U2 Assay Buffer	2 x 10 ml	-20°C
82759	4x U2 Detection Buffer	2 x 2 ml	-80°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	Catalog #
AlphaLISA® Anti-Flag Acceptor Beads	Perkin Elmer #AL112C
AlphaScreen® Glutathione Donor Beads	Perkin Elmer #6765300
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.

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 MDM2-p53 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 lack these components, do not affect AlphaScreen assays.

Assay Protocol

- All samples and controls should be tested in triplicate.
- 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 using Idasanutlin (#82074) or Nutlin-3 (#27711) 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.

- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).
- The protocol described below is designed for MDM2-directed inhibitors and has an MDM2 preincubation step. If p53-directed inhibitors are being tested, consider pre-incubating the inhibitor with p53 instead of MDM2.

Step 1:

1. Thaw **MDM2** on ice. Briefly spin the tube to recover its full content.

2. Dilute MDM2 in U2 Assay Buffer to 0.8 ng/µl (4 µl/well).

3. Prepare the Test Inhibitor (2 µl/well): for a titration prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 10 µl.

   3.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in the U2 Assay Buffer, 5-fold more concentrated than the desired final concentrations. For the positive and negative controls, use U2 Assay Buffer (Diluent Solution).

   OR

   3.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in DMSO, then dilute the inhibitor 20-fold in U2 Assay Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

   Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in U2 Assay Buffer to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 5% DMSO in U2 Assay Buffer (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 4 µl of diluted MDM2 to each well designated “Positive Control” and “Test Inhibitor”.

5. Add 2 µl of Test Inhibitor solution to each well designated “Test Inhibitor”.

6. Add 2 µl of Diluent Solution to the “Positive Control” and “Blank”.

7. Add 8 µl of U2 Assay Buffer to the “Blank”.

8. Pre-incubate the reaction at Room Temperature (RT) for 30 minutes.

Note: The protocol described is designed for MDM2 inhibitors, if p53 binding inhibitors are being tested consider pre-incubating p53 with the inhibitor instead.

9. Thaw **p53** on ice. Briefly spin the tube to recover its full content.

10. Dilute p53 to 0.1 ng/µl in U2 Assay Buffer (4 µl/well).

11. Add 4 µl of p53 to each well designated “Positive Control” and “Test Inhibitor”.

12. Incubate plate at RT for 1 hour.

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

Component	Test Inhibitor	Blank	Positive Control
Diluted MDM2 (0.8 ng/µl)	4 µl	-	4 µl
U2 Assay Buffer		8 µl	
Test Inhibitor	2 µl	–	–
Diluent Solution	–	2 µl	2 µl
30 minutes pre-incubation at RT			
Diluted p53 (0.1 ng/µl)	4 µl	-	4 µl
Total	**10 µl**	**10 µl**	**10 µl**

Step 2:

1. Dilute 4-fold the 4X U2 Detection Buffer with distilled water. This makes 1X Detection Buffer.

2. Dilute 250-fold the Anti-Flag Acceptor Beads with 1x Detection Buffer (10 µl/well).

3. Add 10 µl of diluted Anti-Flag Acceptor Beads into each well.

4. Incubate at RT for 30 minutes.

Step 3:

1. Dilute 125-fold the Glutathione Donor Beads with 1x Detection Buffer (10 µl/well).

2. Add 10 µl of diluted Glutathione Donor Beads into each well.

3. Incubate at RT for 30 minutes.

4. Read Alpha-counts on an AlphaScreen® microplate reader.

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

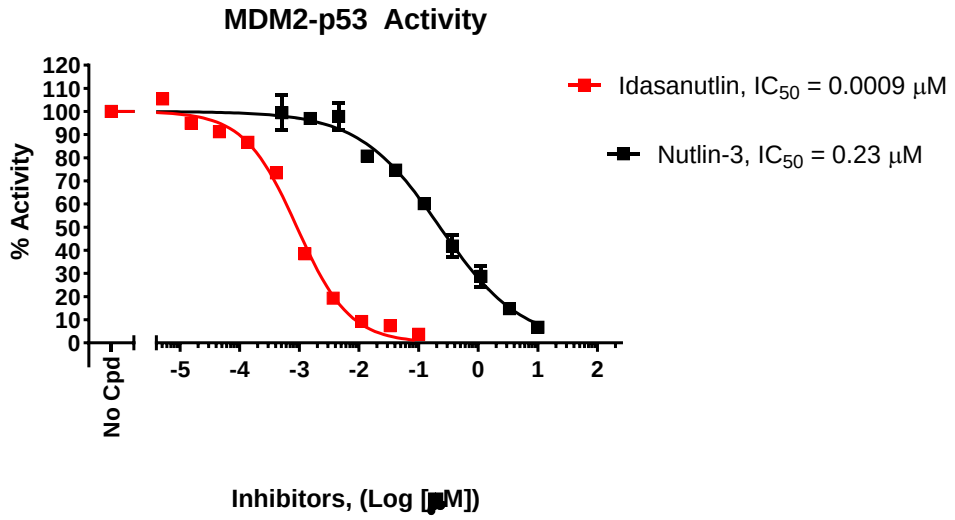

Figure 2: Inhibition of MDM2-p53 binding by Idasanutlin and Nutlin-3.
MDM2-p53 binding activity was measured in the presence of increasing concentrations of Idasanutlin (#82074) or Nutlin-3 (#27711). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (MDM2-p53 binding in the absence of inhibitor, set at 100%).

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

Products	*Catalog #*	*Size*
MDM2-Driven p53 Ubiquitination Assay Kit	82179	384 reactions
MDM2 TR-FRET Assay Kit	79773	384 reactions
MDM2 Intrachain TR-FRET Assay Kit	78302	384 reactions
p53 Luciferase Reporter Lentivirus	78666	500 µl x 2
p53 Luciferase Reporter HCT116 Cell Line	78681	2 vials

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