Description

The Diablo:XIAP Inhibitor Screening Assay Kit is an ELISA-based assay designed to measure the binding between Diablo (also named Smac) and E3 ubiquitin-protein ligase XIAP (X-linked inhibitor of apoptosis, also known as mammalian IAP homolog A) for screening and profiling applications. The Diablo:XIAP Inhibitor Screening Assay Kit comes with enough purified Diablo (amino acids 56-239) and XIAP proteins, primary and secondary antibody, assay buffer, and detection reagent for 100 enzyme reactions.

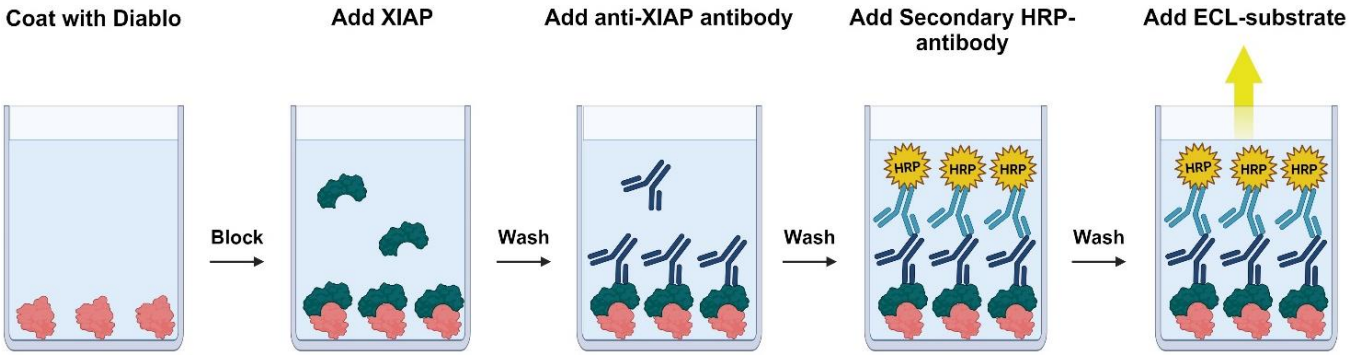

Figure 1. Diablo:XIAP Binding Chemiluminescent Assay Kit schematic.
A 96-well plate is coated with purified Diablo protein. After coating and blocking, XIAP is added in an optimized assay buffer. Next, unbound XIAP is washed away and the plate is incubated with a primary antibody, followed by a secondary HRP-conjugated antibody. Finally, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the binding of XIAP to Diablo.

Background

The XIAP (X-linked inhibitor of apoptosis protein), also known as IAP3 or BIRCA4) protein is a RING-containing E3 Ub ligase which can directly regulate caspases and suppress apoptotic cell death pathways. It is considered the most potent member of the IAP family of proteins. It is composed of three BIR (baculovirus IAP Repeat) domains, a UBA and RING domain. It inhibits caspases 3, 7 and 9 by binding to them and targeting them for proteasome degradation via ubiquitination. XIAP can be inhibited by the mitochondrial protein Diablo, also known as Smac (second mitochondrial derived activator of caspases). Diablo is released from the mitochondrial inner membrane space in a caspase-dependent mode, hours after an apoptotic stimulus. An increased expression level of XIAP has been observed in many cancer types and is associated with cancer cell migration by evasion of apoptosis, while mutations in this protein can result in inflammatory bowel disease or X-linked lymphoproliferative disease type 2. The development of Diablo mimetics has been an active area of research, and the use of these molecules alone or in combinatory therapy may prove beneficial in cancer therapy.

Applications

Screen inhibitors of Diablo:XIAP binding in high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
101118	Diablo, His-Tag*	2 µg	-80°C
80401	XIAP, FLAG-Tag*	1 µg	-80°C
82740	Diablo Binding Assay Buffer	2 x 20 ml	-20°C
82741	Primary Antibody AB31	6 µl	-80°C
52130H	Secondary HRP-Labeled Antibody 1	10 µl	-80°C
79743	Blocking Buffer 3	50 ml	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79837	96-well Maxisorp plate	1	Room Temp

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- 1x PBS (Phosphate Buffer Saline) Buffer
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Stability

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 Diablo:XIAP Binding Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration.

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Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control", and "Test Inhibitor" conditions.
- 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 Smac/ Diablo Peptide (56-64), Biotin-labeled (#82521) 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).

Step 1: Coat 96-well plate

1. Thaw Diablo on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute Diablo to 0.4 ng/µl with PBS (50 µl/well).
3. Add 50 µl diluted Diablo to every well except "Blank" wells.
4. Add 50 µl of Blocking Buffer 3 to "Blank" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times using 200 µl of PBST Buffer per well.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Block the wells by adding 200 µl of Blocking Buffer 3 to every well.
9. Incubate at Room Temperature (RT) for 2 hours.
10. Wash the plate three times using 200 µl of PBST Buffer per well.
11. Tap the plate onto clean paper towel to remove the liquid.

Step 2: Binding reaction

1. Dilute XIAP to 0.4 ng/µl (7 nM) with Diablo Binding Assay Buffer (25 µl/well) (final concentration 3.5 nM).
2. Prepare Test Inhibitor titrations (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final well volume is 50 µl.

   2.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using Diablo Binding Assay Buffer.

   For the positive and negative controls, use Diablo Binding Assay Buffer (Diluent Solution).

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OR

2.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in Diablo Binding Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor in Diablo Binding Assay Buffer at 10-fold the desired final concentrations using 10% DMSO in to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

The final concentration of DMSO should not exceed 1%.

3. Add 20 µl of Diablo Binding Assay Buffer to every well.
4. Add 5 µl Test Inhibitor to each well labeled as "Test Inhibitor".
5. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
6. Add 25 µl of diluted XIAP to all wells.
7. Incubate at RT for 1 hour.

	Blank (non-coated wells)	**Positive Control**	**Test Inhibitor**
Diablo Binding Assay Buffer	20 µl	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted XIAP (0.4 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

8. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.
9. Dilute 1000-fold the Primary Antibody AB31 with Blocking Buffer 3 (50 µl/well).
10. Add 50 µl of diluted Primary Antibody AB31 to every well.
11. Incubate at RT with shaking for 45 minutes to 1 hour.
12. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.
13. Dilute 5000-fold the Secondary HRP-Labeled Antibody 1 with Blocking Buffer 3 (50 µl/well).
14. Add 50 µl of diluted Secondary antibody to every well.

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15. Incubate at RT with shaking for 30-45 minutes.

16. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.

17. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

18. Add 100 µl of mix to every well.

19. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.

20. The "Blank" value should be subtracted from all other values.

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).

Example Results

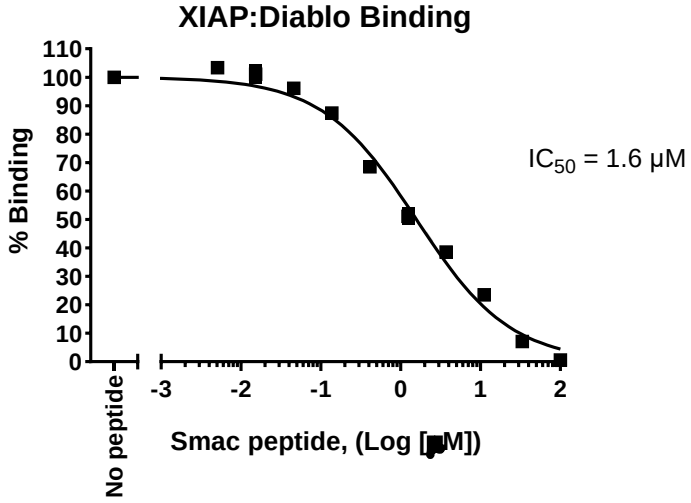

Figure 1. Effect of Smac/Diablo Peptide (54-64), Biotin on XIAP binding to Diablo.
XIAP was incubated with increasing concentrations of Smac/Diablo Peptide (56-64), Biotin (#82521) in a Diablo coated plate. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding in which the condition without Smac peptide is set to 100%.

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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

References

Abbas R. and Larisch S., 2020 *Cells* 9(3): 663.

Related Products

Products	*Catalog #*	*Size*
XIAP Intrachain TR-FRET Assay Kit	78306	384 reactions
XIAP, Bir2-Bir3 Domains, His-Tag Recombinant	75002	50 µg
XIAP (Bir3), His-tag Recombinant	75001	50 µg
Smac/Diablo Peptide (56-64), Biotin	82521	10 µg

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