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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The DCAF5 Intrachain TR-FRET Assay Kit is a homogeneous, sensitive TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer) assay kit designed to measure DCAF5 (DDB1- and CUL4-associated factor 5) complex auto-ubiquitination activity. It utilizes a mix of donor and acceptor labeled ubiquitin to complete the TR-FRET pairing. This assay measures poly-ubiquitination since both the TR-FRET donor and acceptor are incorporated into poly-ubiquitin chains formed on DCAF5, making it suitable for real-time kinetics analyses of poly-ubiquitination. The kit contains enough purified DCAF5 complex, purified UBE1 and UbcH5b, detection reagents and assay buffer for 400 reactions.

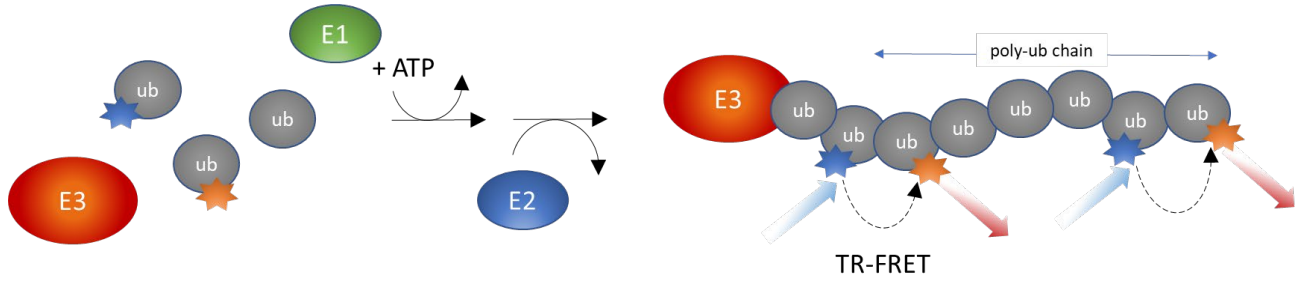

Figure 1: DCAF5 Intrachain TR-FRET Assay Kit schematic.
The TR-FRET signal is proportional to DCAF5 auto-poly-ubiquitination activity.

Background

Covalent conjugation to ubiquitin (Ub) is one of the major post-translational modifications that regulates protein stability, function, and localization. Ubiquitination is the concerted action of three enzymes: a Ub-activating enzyme (E1), a Ub-conjugating enzyme (E2), and a Ub ligase (E3). The specificity and efficiency of ubiquitination are largely determined by the E3 enzyme, which directs the last step of the Ub-conjugating cascade by binding to both an E2~Ub conjugate and a substrate protein. This step ensures the transfer of Ub from E2~Ub to the substrate, leading to its mono- or poly-ubiquitination. DCAF5 (DDB1 and CUL4 associated factor 15) acts as an E3 ubiquitin ligase when complexed with CUL4A (cullin 4A) or CUL4B. DCAF5 is required for cell survival in SMARCB1-mutant cancers. It acts by increasing the degradation of SWI/SNF (Switch/Sucrose Non-Fermentable) complexes when SMARCB1 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1) is absent. The removal of DCAF5 in those cases seems to result in a reverse of the cell to a normal status, and thus targeting DCAF5 may become a promising approach for those types of cancer.

Applications

- Screen molecules that inhibit DCAF5 E3 ligase activity in drug discovery High-Throughput Screening (HTS) applications.
- Determine the IC_{50} of inhibitors of DCAF5 E3 ligase activity.
- Determine DCAF5 E3 ligase activity real-time kinetics.

Supplied Materials

Catalog #	Name	Amount	Storage
80301	UBE1 (UBA1), FLAG-Tag*	25 µg	-80°C
80314	UbcH5b, His-Tag (Human)*	60 µg	-80°C
102471	DCAF5/DDB1/DDA1/Rbx1/ CUL4B Complex*	75 µg	-80°C
82185	200x Ubi-Mix™	40 µl	-80°C
82509	4 mM ATP	2 x 1 ml	-80°C
78856	U2 Assay Buffer	2 x 10 ml	-80°C
79969	White, nonbinding, low volume 384-well microtiter plate	1	Room Temp

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

Materials Required but Not Supplied

- Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
- Adjustable micropipettor and sterile tips
- Orbital Shaker

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

- This kit is compatible with up to 1% final DMSO concentration.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples and controls should be performed in triplicate.
- The assay should include “Blank”, “Positive Control”, “Negative 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 Methylated Ubiquitin Recombinant (#102075) 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).

1. Thaw **UBE1**, **UbcH5b**, **DCAF5 complex**, **U2 Assay Buffer**, **200 x Ubi-Mix™**, and **4 mM ATP** on ice. Briefly spin the tubes to recover their full content.

2. Dilute 200x Ubi-Mix™ 40-fold with U2 Assay Buffer. This makes 5x Ubi-Mix™.

   Note: Do not reuse the diluted solution. 200x Ubi-Mix™ is sensitive to freeze/thaw cycles. Prepare single use aliquots (minimum volume of 5 µl/aliquot) and freeze immediately at -80°C.

3. Dilute **UBE1** to 48 ng/µl with U2 Assay Buffer (400 nM – final concentration in reaction 20 nM) (1 µl/well).

4. Dilute **UbcH5b** to 144 ng/µl with U2 Assay Buffer (8 µM – final concentration in reaction 400 nM) (1 µl/well).

5. Dilute **DCAF5 complex** to 36.4 ng/µl with U2 Assay Buffer (100 nM – final concentration in reaction 25 nM) (5 µl/well).

6. Prepare the Test Inhibitor (4 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 20 µl.

   6.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 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

   6.2 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 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 controls prepare 5% DMSO in U2 Assay Buffer (Diluent Solution) so that all wells contain the same amount of DMSO.

   Note: The final concentration of DMSO should not exceed 1%.

7. Prepare a Deficient Master Mix (15 µl/ "Blank" well): N wells x (4 µl of 5x Ubi-Mix™ + 1 µl of diluted UBE1 + 1 µl of diluted UbcH5b + 4 µl of Diluent Solution + 5 µl of U2 Assay Buffer).

8. Add 15 µl of Deficient Master Mix to the "Blank" wells.

9. Prepare a Master Mix (11 µl/well, except "Blank" wells): N wells x (4 µl of 5x Ubi-Mix™ + 1 µl of diluted UBE1 + 1 µl of diluted UbcH5b + 5 µl of diluted DCAF1 complex).

10. Add 11 µl of Master Mix to all wells, except the "Blank" wells.

11. Add 4 µl of diluted inhibitor to the "Test Inhibitor" wells.

12. Add 4 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

13. Initiate the reaction by adding 5 µl of **4 mM ATP** to the "Blank", "Test Inhibitor", and "Positive Control" wells.

14. Add 5 µl of U2 Assay Buffer to the "Negative Control" wells.

15. Read the fluorescent intensity in a microtiter-plate reader capable of measuring TR-FRET in kinetic mode for up to 60 minutes with intervals of 5 minutes. An end point readout can be done in 40-50 minutes.

	Blank	**Test Inhibitor**	**Positive Control**	**Negative Control**
Deficient Master Mix	15 µl	-	-	-
Master Mix	-	11 µl	11 µl	11 µl
U2 Assay Buffer	-	-	-	5 µl
Test Inhibitor	-	4 µl	-	-
Diluent Solution	-	–	4 µl	4 µl
4 mM ATP	5 µl	5 µl	5 µl	-
Total	**20 µl**	**20 µl**	**20 µl**	**20 µl**

Instrument Settings

Two sequential measurements should be conducted. Eu- donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm.

Reading Mode	Time Resolved
Excitation Wavelength	317 (20 nm bandwidth)
Emission Wavelength	620 (10 nm bandwidth)
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	317 (20 nm bandwidth)
Emission Wavelength	665 (10 nm bandwidth)
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS

Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

$$FRET = \frac{S_{665}}{S_{620}}$$

When percentage activity is calculated, the FRET value from the Blank (it is expected that Blank and Negative Control have a similar values) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\%\ Activity = \frac{FRET_S - FRET_{blank}}{FRET_P - FRET_{blank}} \times 100\%$$

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{blank}$ = FRET value for the Blank, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

Example Results

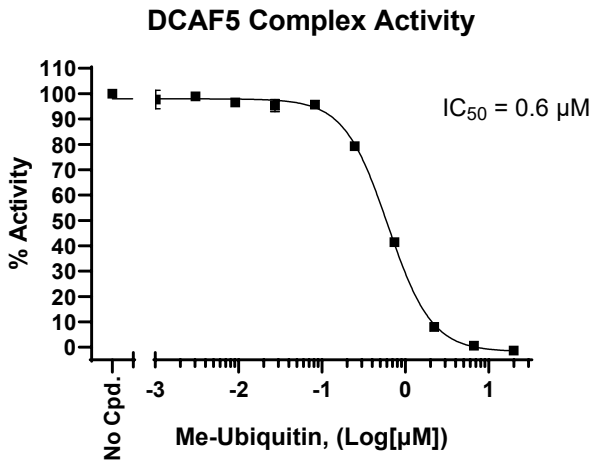

Figure 2: Inhibition of DCAF5 auto-ubiquitination activity by Methylated Ubiquitin Recombinant. DCAF5 auto-ubiquitination was measured in presence of increasing concentrations of Methylated Ubiquitin Recombinant (#102075). Results are expressed as percent activity, in which absence of inhibitor is set to 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

References

Radko-Juettner S., *et al.*, 2024 *Nature* 628: 442-449.

Related Products

Products	*Catalog #*	*Size*
DCAF11 Intrachain TR-FRET Assay Kit	78542	384 reactions
DCAF15 Intrachain TR-FRET Assay Kit	78543	384 reactions
DCAF1 Intrachain TR-FRET Assay Kit	82517	384 reactions
MDM2 Intrachain TR-FRET Assay Kit	78302	384 reactions
SMURF1 Intrachain TR-FRET Assay Kit	78303	384 reactions
VHL Intrachain TR-FRET Assay Kit	78305	384 reactions

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