100x TRF-Ubiquitin Mix (400 wells)

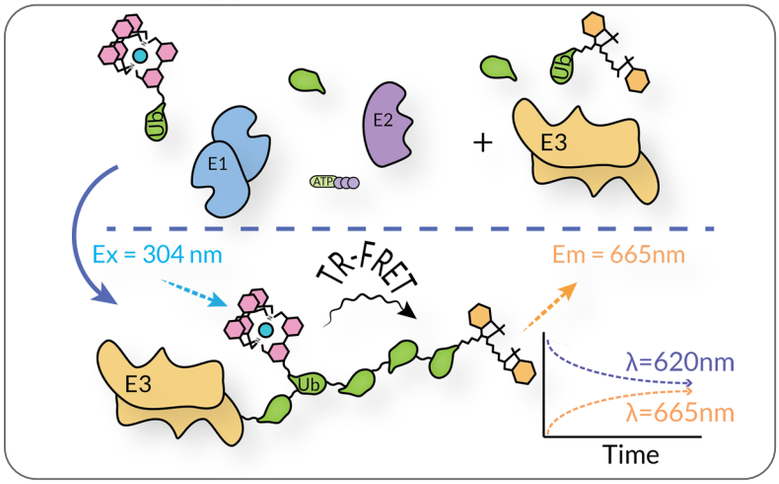
Cat. No. SBB-TR0051-4H
Lot. No. 220150051

South Bay Bío

Introduction

Ubiquitin is a highly conserved protein that plays a major role in the ubiquitination pathway, which is conserved from yeast to mammals. Ubiquitination, the conjugation of ubiquitin to other proteins through a covalent bond between its C-terminal glycine and the ε-amino group of lysine residues (or the ε-amino group of an N-terminal methionine) onto proteins is essential for many cellular process primarily linked to protein degradation. This process involves three steps with specific groups of enzymes in an ATP dependent manner, which are activation with ubiquitin-activating enzymes (E1s), conjugation with ubiquitin-conjugating enzymes (E2s), and ligation with ubiquitin ligases (E3s).

This product is a mix of Europium Cryptate Ubiquitin (SBB-TR0014), Cy5-Ubiquitin (SBB-TR0015), and wild-type Ubiquitin (SBB-UP0013) in a ratio optimized for TR-FRET based conjugation experiments where long polyubiquitin chains are formed. Under conditions where short Ubiquitin chains are formed, a different mix may be required to optimize signal to background ratio. Enzymatic incorporation of the labeled ubiquitins into chains conjugated onto a substrate protein leads to an increase in fluorescence emission at 665 nm ($Em_{acceptor}$) and decrease at emission wavelength 620 nm (Em_{donor}).



Product Information

Quantity: 400 x 20 µL reactions
160 x 50 µL reactions

Excitation/Emission Chanel$_1$= 304nm /620nm
Excitation/Emission Chanel$_2$= 304nm /665nm

Storage: -80C, Avoid multiple freeze / thaw cycles. It is recommended to make per-use aliquots upon first time use.

Example Setup Protocol

1) In this order mix H20, 10x Reaction Buffer, 100x UBA1, 100x E2, 100x E3, 100x trf-UB Mix, and 10x Mg-ATP to a final volume concentration of 1x. Approximate final reaction conditions should contain 10-50nM UBA1, 0.5-1 µM E2, 20-200nM E3, 1-10mM Mg-ATP, but you will need to empirically determine optimal conditions for your assay. Wait to initiate the reactions with Mg-ATP until the plate is ready to read in plate reader.

Example setup for 1 mL final volume mixture:

To	760 µL H20
Add...	10 µL 100x UBA1
	10 µL 100x E2
	10 µL 100x E3
	10 µL 100x TRF-Ub-mix
	100 µL 10x Reaction Buffer

Initiate reaction(s) with final addition of 10% final volume, 10x Mg-ATP to each well. For negative control(s) wells substitute Mg-ATP with 1x Reaction Buffer.

It is recommended to pipette all the components (minus Mg-ATP), i.e. 90% of the final volume into each well first, then initiate the reactions with addition of the last 10% final volume of Mg-ATP or a solution of 1x Reaction Buffer for the negative control wells.

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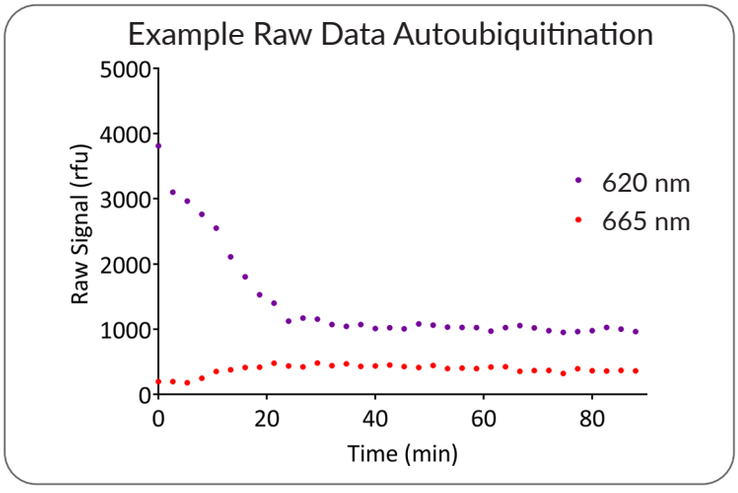
2) Read 20-50µL reactions in a 384-well white low volume plate. You must use a plate-reader capable of taking top-read duel-channel TR-FRET measurements. Plate-readers tested with this kit include the Molecular Devices M5e Plate Reader and the PerkinElmer EnVision™ Multilabel Plate Reader. Plates can be read in either endpoint or continuous kinetic read mode.

Recommended Setting for Molecular Devices M5e
Channel 1: Excitation λ =304nm; Emission λ =620nm
Channel 2: Excitation λ =304nm; Emission λ =665nm
Delay Time = 50µs; Integration Time = 400µs
Cutoff λ_1 =570nm; Cutoff λ_2 =630nm

Recommended Setting for PerkinElmer EnVision
Top Mirror: LANCE/DELFIA Dual Enhanced (#662)
Excitation Filter = UV2 (TRF) 320nm (#111); Emission Filter$_1$ = APC 665nm (#205); Emission Filter$_2$ = Europium 615nm (#203); Delay Time = 50µs; Window Time = 400µs

Raw Data Output: Endpoint & Kinetic

Regardless of which type of readout that has been selected, the data must be collected on two channels; Channel 1 measuring the emission signal of the donor cryptate at λ_1= 620 nm, and Channel 2 measuring the emission of the acceptor cyanine5 at λ_2= 665nm. Both emission channels are required during data reduction to calculate percent signal : background and Z-primes.



Raw Data Output: An example using readout showing a single positive well continuously read in kinetic mode.

Data Reduction

Raw Signal: Aggregate TR-FRET signal detection is most typically quantified as the product of the ratio of acceptor to donor emission signal (λ =665 nm/ λ =620 nm), and a "convenience constant" (η=10^4):

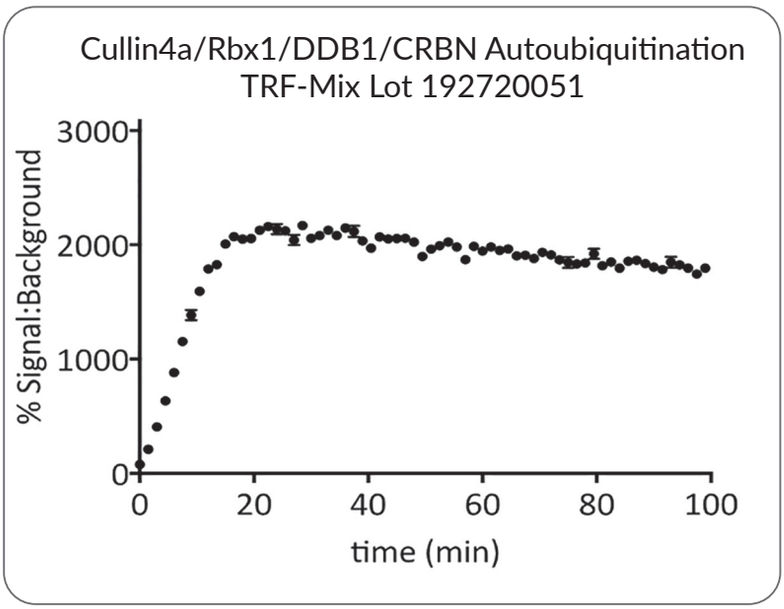
$$\frac{\text{Emission}_2}{\text{Emission}_1}\left(\frac{\lambda=665}{\lambda=620}\right) \times 10^4 = \text{Raw Signal or r(Signal)}$$

Specific Signal or Delta Signal: Subtracting the background raw-signal from the positive experimental-signal yields the "true" signal, also called the Delta Signal:

r(Experimental)-r(Background) = r(Delta)

% Signal to Background: Different plates or experiments from different days of the same assay can be compared using the Signal to Background:

$$\frac{\text{r(Experimental)-r(Background)}}{\text{r(Background)}} \times 100\% = \frac{\text{Signal}}{\text{Background}}\%$$



% Signal to Background of Continious Real-Time TR-FRET Cullin4a/Rbx1/DDB1/CRBN (autoubiquitination): An example of Cullin4a/Rbx1/DDB1/CRBN at 50nM mixed with UBA1, UBE2D1, and, trf-Ub mix. Reaction was initiated with addition of Mg-ATP.

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Z-Prime (Z'): Z-primes can be calculated by using the means and standard deviations of the positive and negative signal at each data-point over time.

$$\text{Estimated Z-prime} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}$$

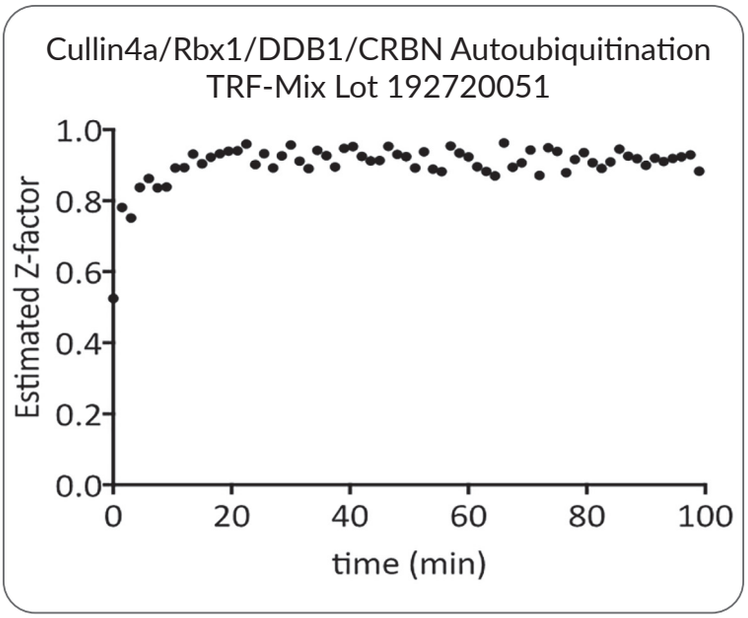
Where :

σ_p = standard deviation of the positive signal.

σ_n = standard deviation of the negative signal.

μ_p = mean of the positive signal.

μ_n = mean of the negative signal.



Estimated Z-primes of Continious Real-Time TR-FRET Cullin4a/Rbx1/DDB1/CRBN (autoubiquitination): An example of Cullin4a/Rbx1/DDB1/CRBN at 50nM mixed with UBA1, UBE2D1, and, trf-Ub mix. Reaction was initiated with addition of Mg-ATP.

References

1) Magennis, S. W., Parsons, S., Pikramenou, Z., Corval, A., & Woollins, J. D. (1999). Imidodiphosphinate ligands as antenna units in luminescent lanthanide complexes. Chemical communications, (1), 61-62.

2) Zheng, N., & Shabek, N. (2017). Ubiquitin Ligases: Structure, Function, and Regulation.Annual Review of Biochemistry, (0).

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