Product Information

Description: The 200x Ubi-Mix™ is a mix of Europium cryptate-labeled Ubiquitin (donor), Cy5-labeled Ubiquitin (acceptor), and unlabeled Ubiquitin at a ratio optimized for TR-FRET based assays where long and/or branched polyubiquitin chains are formed. Under conditions where short ubiquitin chains or multi-monoubiquitylation on a substrate protein are expected, a different reagent might be required.
Since both the TR-FRET donor and acceptor are incorporated into poly-ubiquitin chains, using 200x Ubi-Mix™ avoids time-consuming washing steps and allows for monitoring polyubiquitination real-time kinetics.

Supplied As: Aqueous solution

Formulation: 40 mM Tris-HCl, pH 8.0, 110 mM NaCl, 2.2 mM KCl, and 20% glycerol

Storage: Upon receipt, store at -80°C.

Stability: Stable for 6 months from date of receipt, when stored as directed. Avoid multiple freeze/thaw cycles.

Applications: This product has been optimized for use with the BPS Bioscience Intrachain TR-FRET Assay Kits and U2 Assay Buffer (BPS Bioscience #78856). For a detailed protocol on the use of this product with BPS Bioscience Intrachain TR-FRET Assay Kits, please see the appropriate assay kit protocol. Use in other assays may require optimization.

Instructions for Use: Thaw on ice, perform a quick spin and gently mix by tapping prior to use. DO NOT VORTEX. Single use aliquots can be made after the first thaw (minimum 10 µl aliquots). Do not re-use the diluted mixture.
We recommend to dilute 200x Ubi-Mix™ 200-fold and perform reactions in U2 Assay Buffer (BPS Bioscience #78856). Assay reactions should contain 10-50 nM E1, 100-1000 nM E2, 10-200 nM E3, 1 mM ATP, and 1x Ubi-Mix™. Mix all protein components of the E1/E2/E3 conjugation cascade and the inhibitor/activator of interest (if applicable) prior to adding ATP substrate. Initiate the reactions by adding ATP. For negative controls use U2 Assay Buffer. Reactions can be read as endpoint or in continuous kinetic read mode. Two sequential measurements should be conducted. Donor emission should be measured at 620 nm followed by acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (620 nm emission/665 nm emission).
Recommended instrument settings:

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

Note: The conditions described may require further development to achieve optimal results.

Shipping Temperature: -80°C