

# Fluorescein-Anti 6xHis Monoclonal mAb

Catalog # T6010

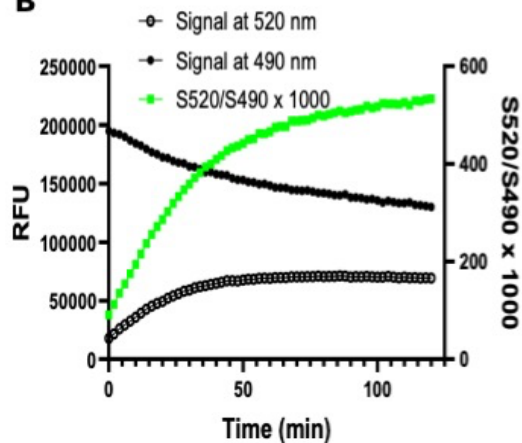
<b>Also Known as:</b>	Fluorescein-Anti-His Antibody
<b>Quantity:</b>	50 µg
<b>Isotype:</b>	Mouse IgG2b
<b>Clone:</b>	His.H8
<b>MW:</b>	150 kDa
<b>Species:</b>	Mouse monoclonal
<b>Immunogen:</b>	HHHHHH (6xHis) synthetic peptide conjugated to KLH
<b>Applications:</b>	WB and TR-FRET
<b>Stock Buffer:</b>	10 mM PBS (pH 7.2), 10% Glycerol, 0.09% NaN <sub>3</sub> (sodium azide)
<b>Concentration:</b>	See tube label
<b>Quality Assurance:</b>	Validated by RELAY <sup>TR</sup> E2-Ub charging assays.

Image

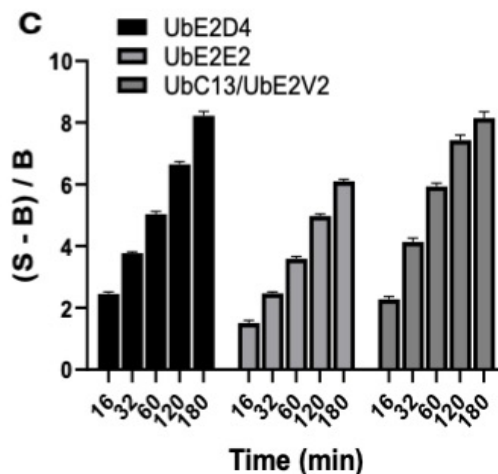
## A RELAY<sup>TR</sup> E2-Ub Charging Assay



## B



## C



A. Schematic of the RELAY<sup>TR</sup> E2-Ub Charging Assay using 6xHis-tagged E2, fluorescein-Anti-6xHIS antibody and terbium-Ub.

B. Kinetic mode monitoring 10 nM 6xHis-Ubc13/Ubc2V2 charging with Ub. S520/S490 ratios from reactions with ATP were positive Signal (S), and without ATP were Backgrounds (B, data not shown).

C. Time course of E2-Ub charging assays. The signal-to-background ratio was calculated by using the formula of (S-B)/B.

**Description:** Fluorescein (6-FAM) is covalently conjugated on anti-His monoclonal mAb (clone #: HIS.H8), and purified by gel filtration. Usually, the molar ratio of fluorescein to antibody is at the range of 3-12.

In TR-FRET assays, a typical concentration of fluorescein labeled antibody is equal or less than the concentration of the target protein. User should optimize the assay to achieve a desirable signal-to-background ratio, including the concentration and ratio of fluorescein or terbium labeled antibody, IgG or proteins corresponding to your specific assay design.

TR-FRET assay reaction time is usually 1-3 hours in kinetic or end point assay.

**Storage:** Store at -80°C; Avoid multiple freeze-thaw cycles

**Note:** A TR-FRET capable plate reader is required. Our assays were performed using a PHERAstar FS instrument with the 337/520/490 nm filter set. Intergration started at 50  $\mu$ s, and intergration time was 400  $\mu$ s.

**Literature:** <https://www.bmglabtech.com/en/tr-fret/>