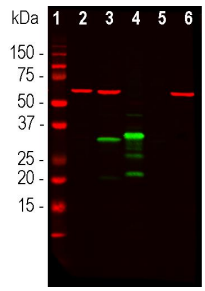
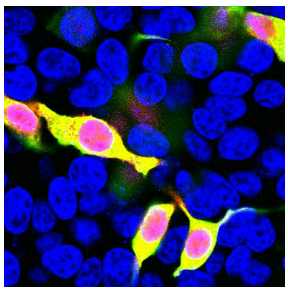
Product Data Sheet: td-Tomato

Cat. No:	AB-84342
Conjugate:	Unconjugated
Size:	100 ug
Clone:	POLY
Concentration:	1mg/mL
Host:	Rb
Isotype:	IgG
Immunogen:	Full length recombinant td-Tomato protein
Reactivity:	All Species
Applications:	Western Blot: 1:500 Immunofluorescence: 1:250 Immunohistochemistry: 1:250
Molecular Weight:	28kDa
Purification:	Aff. Pur.
Background:	td-Tomato is derived from proteins originally isolated from Cnidarians (jelly fish, sea anemones and corals), and is used as a fluorescent tracer in trasfection and transgenic experiments. The prototype for these fluorescent proteins is Green Fluorescent Protein (GFP), which is a ~27 kDa protein isolated originally from the jellyfish Aequoria victoria. GFP was the basis of the 2008 Nobel Prize in Chemistry, awarded to Osamu Shimomura, Martin Chalfie and Roger Tsien, specifically “for the discovery and development of the green fluorescent protein, GFP”. GFP was shown to fluoresce on contact with molecular oxygen, requiring no other cofactors, and so can be expressed in fluorescent form in essentially any prokaryotic or eukaryotic cell. The td-Tomato protein is derived from DsRed, a red fluorescent protein related to GFP isolated from so-called disc corals of the genus Discosoma. DsRed is similar in size and properties to GFP, but, obviously, produces a red rather than a green fluorochrome. The original DsRed was engineered extensively in the Tsien lab to prevent it from forming tetramers and dimers and to modify and improve the spectral properties (1-3). Several further cycles of mutation, directed modification and evolutionary selection produced td-Tomato, which has an excitation maximum at 587 nm and and emission maximum at 610 nm (4).We expressed the td-Tomato protein sequence shown in reference 4 in bacteria, purified out the td-Tomato and raised this rabbit polyclonal antibody. This was affinity purified and was found to stain a band of the expected size in HEK293 cells transfected with the pFin-EF1-td-Tomato vector designed to express td-Tomato which was obtained from Clontech. As shown below, the antibody does not stain any protein band in untransfected HEK293 cells.
Form:	Liquid
Buffer:	Affinity purified antibody at 1 mg/mL in 50% PBS, 50% glycerol plus 5mM NaN3
Storage:	Shipped on ice. Store at 4°C. For long term storage, leave frozen at -20°C. Avoid freeze / thaw cycles



Western blot analysis of HEK293 cell lysates, and recombinantprotein solutions using rabbit pAb to td-Tomato, td-Tomato,dilution 1:1,000, in green [1] protein standard, [2] HEK293, [3]HEK293 cells transfected with td-Tomato-HA construct, [4] td-Tomato recombinant protein, [5] GFP recombinant protein, and [6] EK293transfected with a full length GFP construct. A major band at about28kDa corresponds to td-Tomato protein. The td-Tomato antibody does not react with GFP protein. The same blot was simultaneously probed with chicken pAb to HSP60, dilution 1:5,000 in red which reveals a band at 60kDa only in cell lysates.



Immunofluorescent analysis of HEK293 cells transfected with td-Tomato-HA, construct, in red, and stained with rabbit pAb to td-Tomato, td-Tomato, dilution 1:1,000, in green. The blue is Hoechst staining of nuclear DNA. td-Tomato antibody reveals td-Tomato protein expressed only in transfected cells which appear golden in color. Untransfected cells do not react with the antibody, as a result only their nuclei are visible.

References

1. Baird GS, Zacharias DA, Tsien RY. Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. Proc Natl Acad Sci U S A. 97:11984-9 (2000).
2. Gross LA, Baird GS, Hoffman RC, Baldridge KK, Tsien RY. The structure of the chromophore within DsRed, a red fluorescent protein from coral. Proc Natl Acad Sci U S A. 97:11990-5 (2000).
3. Heikal AA, Hess ST, Baird GS, Tsien RY, Webb WW. Molecular spectroscopy and dynamics of intrinsically fluorescent proteins: coral red (dsRed) and yellow (Citrine). Proc Natl Acad Sci U S A. 97:11996-2001 (2000).
4. Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. Nature Biotechnology 22:1567-1572 (2004).