NH DyeAGNOSTICS

T-Dye Series

Product Guide

T-Green 210 Protein Labeling Kit
T-Red 310 Protein Labeling Kit
T-Rex 330 Protein Labeling Kit
T-Rex 410 Protein Labeling Kit

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T-Dye Protein Labeling Kit

The provided T-Dye is a NHS ester activated fluorescent chromophore for easy binding to lysine residues of proteins. Due to the excellent fluorescence properties of the T-Dyes up to 0.15 ng (T-Rex 330: 0.05 ng) of labeled protein is detectable using appropriate detection systems.
T-Dye labeled proteins are compatible to post-stains like silver nitrate or Coomassie Blue as well as to analyses by mass spectrometry.
This labeling protocol allows quantitative 1D- and 2D gel analyses since the labeling is restricted to 3% of all contained proteins, i.e. one label per protein.

Kit contents

- **20 vials containing T-Dye fluorescent dye each vial sufficient for labeling of 50 µg of protein**
- **4 vials containing T-Dye solvent each sufficient for at least 20 labeling reactions**

Note

The T-Dye solvent contains dimethylformamide (DMF, $HCON(CH3)_2$ CAS No: 68-12-2) and is harmful upon inhalation, ingestion, or skin contact.

1 2 3 Easy & fast protein labeling protocol

In order to keep pipetting losses to a minimum we recommend to use Low Retention Mass Spec compatible pipette tips and micro-centrifuge tubes (e.g. NH DyeAGNOSTICS product no. PR052).

1 Sample preparation

Dissolve your protein in a suitable sample buffer. For accurate fluorescence signal quantification (ratio fluorescence signal/amount of labeled protein) a protein concentration between 1 to 5 µg/µl for labeling is required. However, protein labeling with a protein concentration between 0.1 to 15 µg/µl can be performed.

Recommended sample buffer

For efficient labeling a pH > 8.0 of the buffer and the absence of primary amines are crucial. The labeling compatibility of the following buffer compounds has been tested.

compound	compatible
Tris	yes (≤ 100 mM)
HEPES	yes (≤ 100 mM)
Phosphate	yes (≤ 100 mM)
Urea	yes (≤ 8 M)
Thiourea	yes (≤ 2 M)
SDS	yes (≤ 5 %)
Triton X-100	yes (≤ 1 %)
CHAPS	yes (≤ 4 %)
DTT	no
Mercaptoethanol	no
EDTA	yes (≤ 5 mM)
NaCl	yes (≤ 150 mM)
KCl	yes (≤ 50 mM)
Glycerol	yes (≤ 15 %)
Sucrose	yes (≤ 12 %)
Amino acids	no
Bromphenol blue	no
Protease inhibitor cocktail (Sigma Aldrich)	yes (≤ 1 %)

To check for labeling compatibility of other compounds or compound concentrations, we recommend the labeling of 50 µg standard protein (e.g. BSA) using 30 mM Tris-HCl (pH 8.5) as basic buffer and the novel compound. Use as a positive control for labeling a 30 mM Tris-HCl (pH 8.5) buffer without the compound. Separate 0.25 µg of the labeled protein by 1D SDS-PAGE and check for the fluorescence signal.

Recommendations for 1D SDS-PAGE and other applications

If your analysis requires the reduction of protein, reduce protein after labeling or remove reducing agents before labeling by dialysis or gel filtration. For 1D SDS-PAGE add bromphenol blue or reducing agents containing PAGE loading buffer after the labeling step.

Recommended sample buffer (for 2D gels)

Since 2D sample buffers must be compatible to the protein labeling as well as to isoelectric focusing (IEF), we recommend the use of the following 2D sample buffer.

compound	concentration	quantity
Tris	30 mM	0.18 g
Urea	7 M	21.00 g
Thiourea	2 M	7.60 g
CHAPS	4% (w/v)	2.00 g
add deionized water to a total volume of 50 ml; adjust pH to 8.5		

Do not heat buffer to dissolve urea. Store in small aliquots at -20°C.

2 Protein labeling

1. Allow the T-Dye vial to warm up to ambient temperature (ca. 5 min).
2. Centrifuge briefly.
3. Dilute the T-Dye in 2 µl T-Dye solvent.
4. Mix (Vortex) and spin down briefly.
5. Transfer 50 µg of protein sample (ideally up to 10 µl, 20 µl maximum) into the vial.
6. If necessary, add sample buffer until a total volume of 12 µl.
7. Mix (Vortex) and spin down briefly.
8. Incubate the reaction mixture for 30 min on ice.
9. The protein sample is now labeled with T-Dye, and ready to use for downstream applications.

3 Detection

For the detection of T-Green 210 use the recommended filter settings of your imaging system for G-Dye200, Cy3 or Alexa 555.

For the detection of T-Red 310 and T-Rex 330 use the recommended filter settings of your imaging system for G-Dye300, Cy5 or Alexa 647.

For the detection of T-Red 410 use the recommended filter settings of the OCTOPLUS Fluorescence Imager or for Alexa 700.

In order to get the optimum fluorescence performance of the T-Dye we recommend a pre-scan for each gel in order to determine the optimum exposure time of your camera or the optimum detection voltage of your scanner. The signal of the strongest protein bands or protein spots should be marginally **below** saturation.

Excitation and emission properties

	excitation max. [nm]	emission max. [nm]
T-Green 210	559	585
T-Red 310	650	665
T-Rex 330	650	665
T-Rex 410	705	722

Storage of labeled protein gels

Acquire the fluorescent image of the T-Dye labeled protein gels immediately after finishing the SDS-PAGE.
Gels stored within low fluorescent glass cassette (product no PR03 and PR04) can be scanned up to 6 h after finishing the SDS-PAGE.
Otherwise, fix the gel for 30 min in 40% ethanol / 10% acetic acid and store in 25% Ethanol / 3% glycerol in the dark.
For pre-cast gels, please see manufacturers' recommendations.

Storage of T-Dyes

Store the T-Dye containing vials protected from light and dry at -20°C to -80°C.
Labeled proteins can be stored for up to three months at -80°C until further use.

Best before: see packaging.