NH DyeAGNOSTICS

Product Guide

T-Rex Protein Labeling Kit

Product no. PR06

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FOR RESEARCH USE ONLY

1 Products and content

- 20 vials containing T-Rex fluorescent dye each vial sufficient for labeling of 50 µg of protein
- 4 vials containing T-Rex solvent

2 Storage and stability

Store T-Rex and T-Dye Solvent dark at -20°C to -80°C.

Best before: see kit package

Short-term storage (< 2h) of dissolved T-Dyes: +2 to +8°C. Long-term storage of dissolved T-Dyes: -20 to -80°C. Use dissolved T-Dyes within 3 weeks. Avoid repeated freeze-thaw-cycles. Store labeled protein at -20°C to -80°C. Labeled protein can be stored at least for 3 month.

3 Safety instructions

The T-Dye Solvent contains dimethylformamide (DMF, $HCON(CH_3)_2$, CAS No: 68-12-2) and is harmful by inhalation, ingestion or skin contact.

4 Additional materials required:

- 2D gel electrophoresis system (incl. required material)
- opt.: Low-fluorescent glass cassette or VELUM GOLD Precast 2D Gels (PR237, PR241)
- Imaging system for detection of blue, green and red
- Software for data evaluation (e.g. Delta 2D; available at www.dyeagnostics.com)

5 General Information

The provided T-Rex is a NHS ester activated fluorophor binding to lysine residues of proteins. Due to the excellent fluorescence properties of T-Rex down to 0.05 ng of T-Rex labeled protein is detectable (using appropriate imaging device). T-Rex labeled proteins are compatible to post-stains like silver nitrate or Coomassie Blue as well as to analyses by mass spectrometry.

6 Detailed protocol for T-Rex Labeling

6.1 Sample preparation

Dissolve your protein in a suitable sample buffer. For fluorescence signal quantification (ratio fluorescence signal vs.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.

6.1.1 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	yes (≤ 5 mM)
Mercaptoethanol	yes (≤ 1%)
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 no-vel 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.

6.1.2 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.

6.1.3 Recommended buffer for T-Rex labeling and 2D gel electrophoresis

Do not heat! Store aliquots at -20°C to -80°C lagern.

reagent	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		

6.2 Protein labeling

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

6.3 Detection

Imaging parameters (e.g. voltage of the photomultiplier tube (PMT) or exposure time of the CCD camera) are dependent on the fluorophore, the gel quality and constitution of the sample. For best fluorescence performance optimize detection parameters for each dye by imaging the gel with a low resolution scan. Signal intensity of the most abundant spot(s) should be marginally below saturation (saturation: 65,535 grey values for 16 bit).

Acquire the fluorescent image of the T-Dye labeled protein gels after finishing SDS-PAGE.

Please find further information on www.dyeagnostics.com/

T-Rex excitation and emission parameter

	max. excitation [nm]	max. emisson [nm]
T-Rex	650	665

8 Post-electrophoretic applications

Gels stored within low fluorescent glass cassettes (product no PR03 and PR04) can be imaged up to 24 h after finishing SDS-PAGE. Otherwise, fixate the gel for 30 min in fixing solution (40% ethanol/ 10% acetic acid) and than store the gel in a solution containing 25% ethanol/ 3% glycerol in the dark (incubate for 15 min in water before scanning). For pre-cast gels see manufacturers' recommendations.

T-Dye label does not interfere with protein identification by mass spectrometry, enymatic digestions or sequence coverage.

T-Dye labeled proteins can be blottet and stained with common stains (note: observe detection limits as well as excition and emission parameters of the stains; post-electrophoretic stains may mask T-Dye fluorescence signals).