TECHNICAL BULLETIN

# FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System

Instructions for Use of Product
**L5001**


Revised 12/17
TB285

# FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System

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## 1. Description

The FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System[a] allows fluorescent labeling of in vitro translation products through the use of a modified charged lysine transfer RNA labeled with the fluorophore BODIPY®-FL. Using this system, fluorescently labeled lysine residues are incorporated into nascent proteins during translation, eliminating the requirement for labeling with [$^{35}$S] methionine or other radioactive amino acids. The fluorescent lysine is added to the translation reaction as a charged epsilon-labeled fluorescent lysine-tRNA complex (FluoroTect™ Green$_{Lys}$ tRNA) rather than a free amino acid (Figure 1).

Synthesized proteins are resolved by conventional SDS-PAGE analysis. Following gel electrophoresis, detection of the labeled proteins is accomplished in 2–5 minutes directly "in-gel" using a laser-based fluorescent scanner. This eliminates any requirements for protein gel manipulation associated with the use of radioactively labeled amino acids such as fixing/drying or overnight exposure to X-ray film. The convenience of in-gel detection also avoids the time-consuming electroblotting and detection steps of conventional nonisotopic detection systems (Figure 2).

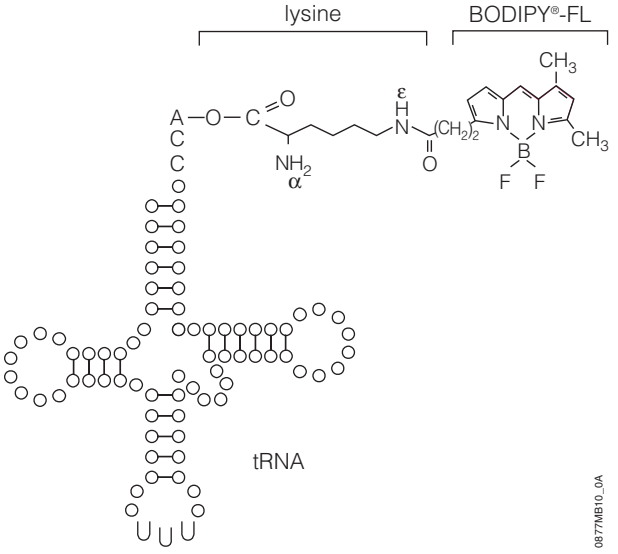


**Figure 1. Structure of FluoroTect™ Green$_{Lys}$ tRNA.**

The use of the FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System offers several advantages:

- **Fast:** Data can be obtained in minutes, eliminating overnight exposures associated with radioactivity-based systems or time-consuming steps used by traditional nonisotopic methodologies.
- **Convenient:** Results based on in-gel detection. No requirement to transfer, fix or dry gels.
- **Non-Radioactive:** No safety, regulatory or waste disposal issues associated with radioactivity.
- **Flexible:** The modified charged tRNA can be used with all Promega translation systems.
- **Sensitive:** Comparable to traditional radioactive and non-radioactive labeling methods.

The charged *E. coli* lysine tRNA provided in the FluoroTect™ System is chemically labeled with the fluorophore BODIPY®-FL at the epsilon amino group using a modification of the methodology developed by Johnson *et al.* (1). The resulting fluorescently labeled lysine tRNA molecule (FluoroTect™ Green$_{Lys}$ tRNA) can be used in eukaryotic or prokaryotic in vitro translation systems such as the TnT® Quick Coupled Transcription/Translation Systems, Rabbit Reticulocyte System, Wheat Germ Extract or *E. coli* S30 Extract System. Lysine is one of the more frequently used amino acids. On average, lysine represents 6.6% of a protein's amino acid content, whereas methionine represents only 1.7% (2).

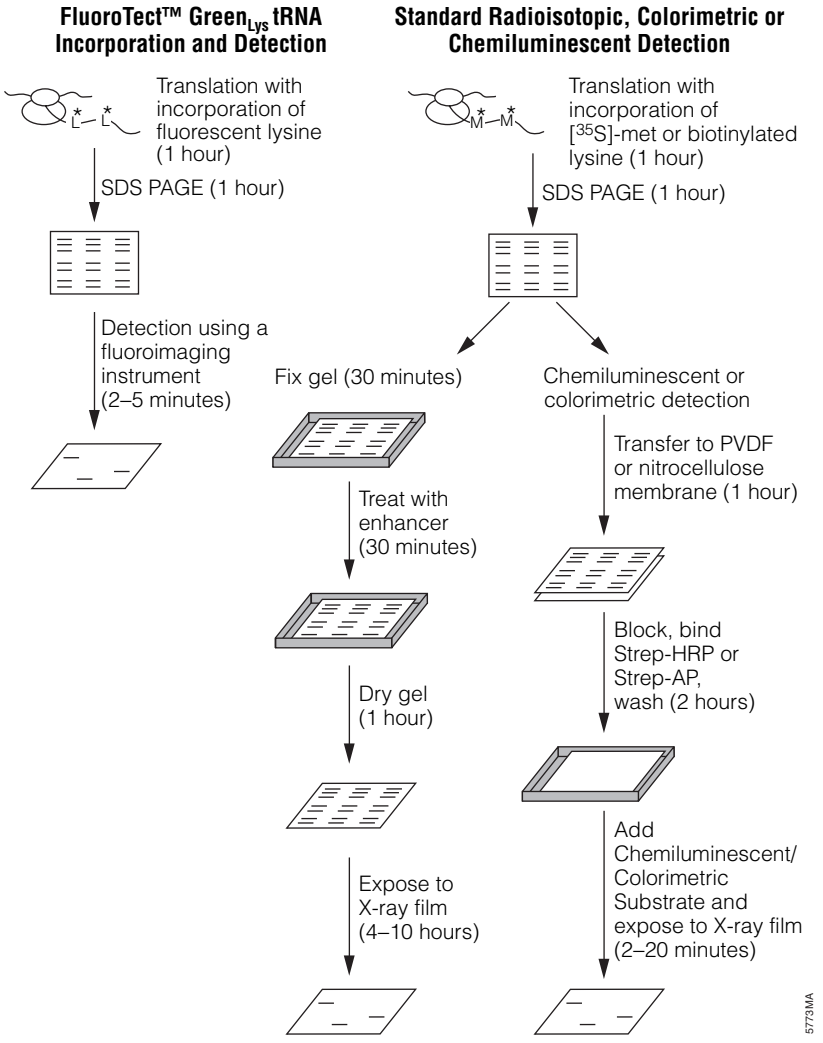


**Figure 2. Comparison of incorporation and detection protocols using FluoroTect™ Green$_{Lys}$ tRNA and radioactive or nonisotopic labeling methods.**

## 2. Product Components and Storage Conditions

| Product | Size | Cat.# |
|---|---|---|
| FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System | 40 reactions | L5001 |

Each system contains sufficient reagents to label 20–40 translation reactions. Includes:

- 40µl FluoroTect™ $Green_{Lys}$ tRNA

**Storage Conditions:** Store at –70°C. Product is sensitive to $CO_2$ (avoid prolonged exposure) and multiple freeze-thaw cycles, which may have an adverse effect on activity/performance. Do not subject the FluoroTect™ $Green_{Lys}$ tRNA to more than 5 freeze-thaw cycles. If necessary, store in multiple aliquots at –70°C.

## 3. Protocol: Fluorescent Lysine Incorporation Using FluoroTect™ $Green_{Lys}$ tRNA

The FluoroTect™ $Green_{Lys}$ tRNA is labeled with BODIPY®-FL, which has an excitation maximum of 502nm and an emission maximum of 510nm. The BODIPY®-FL fluorophore is compatible with widely used excitation sources and common optical filter sets.

### Materials to Be Supplied by the User

- Nuclease-Free Water (Cat.# P1193)
- translation system (e.g., TNT® Coupled Transcription/Translation System [see Note 1], Rabbit Reticulocyte Lysate [see Note 1], Wheat Germ Extract or *E. coli* S30 Extract)
- complete amino acid mix or a combination of two minus amino acid mixes
- salts, DTT and other components as needed to optimize the translation reaction

Use the following protocol as a guideline to set up translation reactions using FluoroTect™ $Green_{Lys}$ tRNA. In general, FluoroTect™ $Green_{Lys}$ tRNA may be used in in vitro translation protocols at a concentration of 1µl of FluoroTect™ $Green_{Lys}$ tRNA per 50µl reaction. Examples of standard reactions using TNT® T7 Quick for PCR DNA and Rabbit Reticulocyte Treated Lysate are provided.

1. Remove the translation and FluoroTect™ $Green_{Lys}$ tRNA reagents from storage. Thaw the translation lysate and FluoroTect™ $Green_{Lys}$ tRNA by quick hand-warming, and immediately place on ice. The other components can be thawed at 37°C and stored on ice as soon as they are thawed.
2. Set up 50µl translation reactions on ice, as for radioactive amino acid incorporation, with the following exception: Add 1µl of a complete amino acid mix (containing 1mM of each amino acid) or a combination of two minus amino acid mixtures (such as 0.5µl of minus methionine and 0.5µl of minus leucine).

   **Note:** We recommend including a control reaction containing FluoroTect™ $Green_{Lys}$ tRNA but no DNA or mRNA. This allows measurement of any background bands from any endogenous proteins or the charged tRNA that show fluorescence under the same conditions.
3. Add all components except the FluoroTect™ $Green_{Lys}$ tRNA, and gently mix by pipetting the reaction while stirring with the pipette tip. If necessary, spin briefly in a microcentrifuge to return the sample to the bottom of the tube. Add the FluoroTect™ $Green_{Lys}$ tRNA.

**Example Using TnT® T7 Quick for PCR DNA with FluoroTect™ Green$_{Lys}$ tRNA**

| | |
|---|---|
| TnT® T7 PCR Quick Master Mix (Cat.# L5540) | 40µl |
| 1mM methionine | 1µl |
| PCR-generated DNA template | 2.5–5µl |
| FluoroTect™ Green$_{Lys}$ tRNA | 1–2µl |
| Nuclease-Free Water to a final volume of | 50µl |

**Example Using Rabbit Reticulocyte Lysate with FluoroTect™ Green$_{Lys}$ tRNA**

| | |
|---|---|
| Rabbit Reticulocyte Lysate, Treated (Cat.# L4960) | 35µl |
| RNasin® Ribonuclease Inhibitor | 1µl |
| Amino Acid Mixture, Complete | 1µl |
| FluoroTect™ Green$_{Lys}$ tRNA | 1–2µl |
| Luciferase Control RNA | 1µl |
| Nuclease-Free Water to a final volume of | 50µl |

4. Incubate at 30°C for 60–90 minutes.
5. Terminate the reaction by placing on ice. If necessary, the translation reaction can be stored for several months at –20°C or –70°C.

**Notes:**

1. In rabbit reticulocyte lysate, there is a 30kDa endogenous fluorescent band. There is also an endogenous fluorescent band from hemoglobin that migrates at or below 12–15kDa.
2. For all systems based on rabbit reticulocyte lysate, an 18kDa endogenous fluorescent band from charged tRNA can be removed by treatment with RNase ONE™ Ribonuclease (5 units/50µl reactions, incubate for 5 minutes at 37°C) or RNase A (4mg/ml) treatment (dilute RNase A at a ratio of 1:10 to 1:20 in water and use 1µl/5µl translation reaction; incubate for 5 minutes at 37°C).
3. Fluorescent labeling of poorly expressed proteins containing few lysines can be increased by adding greater amounts of FluoroTect™ Green$_{Lys}$ tRNA to a 50µl reaction.
4. For maximal expression of your protein, optimize the amount of template added to the reaction, and use highly purified RNA or DNA, depending on the translation system used.
5. The appropriate incubation temperature will vary from one translation system to another. Please refer to the appropriate Promega protocol for specific reaction conditions.
6. No purification is required if a PCR-generated DNA template is used.

## 4. Post-Translational Analysis

Resolve the fluorescent translation product by running a sample on an SDS-PAGE gel, then visualize by placing the gel on a laser-based fluorescence scanning device.

**Note:** The use of gel systems other than Tris-glycine may cause different migration patterns for expressed proteins and background bands.

### 4.A. Denaturing Gel Analysis of Translation Products

1. Once the translation reaction is complete (or at any desired time point), remove a 5µl, and add it to 20µl of 1X SDS sample buffer. Store the remainder of the translation reaction at –20°C. The FluoroTect™ tRNA fluorophore is sensitive to extreme heating. If heating to denature the proteins, do not exceed 70°C for more than 2–3 minutes.
2. Load the sample from Step 1 on an SDS-PAGE gel.
3. Perform electrophoresis using standard conditions for your apparatus. Typically, electrophoresis is carried out at a constant current of 20mA. Electrophoresis is usually performed until the bromophenol blue dye has run off or is near the bottom of the gel.

### 4.B. Fluorescence Detection

**Materials to Be Supplied by the User**

- A laser-based fluorescent imaging instrument is required to ensure sufficient sensitivity for detection (i.e., FluorImager® SI or FluorImager® 595, both with a 488 argon laser; the Typhoon® 8600 [GE Healthcare], with a 532nm excitation, or the FMBIO® II [Hitachi], with a 505 channel)

Immediately after electrophoresis is complete, place the gel in water, then in the fluorescent scanning instrument.

Use gloves when handling the gels.

Instruments that use white light or UV transillumination as the light source should not be used with the FluoroTect™ System (e.g., ChemiDoc™ XRS+ System [Bio-Rad]). These instruments lack the required intesnsity to adequately excite the BODIPY-FL® for sensitive detection (see Note 5).

**Notes:**

1. The STORM® Instrument (GE Healthcare) is not recommended for use with the FluoroTect™ System due to reduced sensitivity.
2. Fixing polyacrylamide gels does not interfere with detection of FluoroTect™ $Green_{Lys}$-labeled in vitro translation products, although the signal intensity may be somewhat decreased.
3. Drying fixed polyacrylamide gels in cellophane does not interfere with the detection of FluoroTect™ $Green_{Lys}$-labeled in vitro translation products, although the signal intensity may be somewhat decreased.
4. Fixing and/or drying gels may decrease the signal intensity of prestained molecular weight markers, making them difficult to detect with fluorescent scanners.

5. Instruments that use Class 1 lasers or LEDs (e.g., STORM® Instrument or LAS 4010 ImageQuant [GE Healthcare]; Fluorchem R [ProteinSimple] are not recommended for use with the FluoroTect™ System due to similar issues with insufficient output intensity that results in reduced detection sensitivity.

### 4.C. Immunoprecipitation and Western Blot Analysis

Anti BODIPY®-FL is available from Invitrogen (Cat.# A-5770) for immunoprecipitation and Western blot analysis of translation products.

## 5. Composition of Buffers and Solutions

**1X SDS gel-loading solution**

| | |
|---|---|
| 50mM | Tris-HCl (pH 6.8) |
| 100mM | dithiothreitol |
| 2% | SDS |
| 0.1% | bromophenol blue |
| 10% | glycerol |

1X SDS gel-loading buffer lacking dithiothreitol can be stored at room temperature. **Dithiothreitol should be added from a 1M stock just before the buffer is used.**

**SDS polyacrylamide running 10X buffer**

| | |
|---|---|
| 30g | Tris base |
| 144g | glycine |
| 100ml | 10% SDS |

Add deionized water to a final volume of 1L. Store at room temperature.

## 6. References


1. Johnson, A.E. *et al*. (1976) N-epsilon-acetyllysine transfer ribonucleic acid: A biologically active analogue of aminoacyl transfer ribonucleic acids. *Biochem*. **15**, 569–75.
2. Dayhoff, M.O. (1978) *Atlas of Protein Sequence and Structure*, Suppl. 2, National Biomedical Research Foundation, Washington.

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## 7. Related Products

### Eukaryotic Transcription/Translation Systems

| Product | Size | Cat.# |
|---|---|---|
| TnT® T7 Quick Coupled Transcription/Translation System | 40 reactions | L1170 |
| TnT® T7 Quick Coupled Transcription/Translation System, Trial Size | 5 reactions | L1171 |
| TnT® SP6 Quick Coupled Transcription/Translation System | 40 reactions | L2080 |
| TnT® SP6 Quick Coupled Transcription/Translation System, Trial Size | 5 reactions | L2081 |
| TnT® T3 Coupled Reticulocyte Lysate System | 40 reactions | L4950 |
| TnT® T7 Coupled Reticulocyte Lysate System | 40 reactions | L4610 |
| TnT® T7 Coupled Reticulocyte Lysate System, Trial Size | 8 reactions | L4611 |
| TnT® SP6 Coupled Reticulocyte Lysate System | 40 reactions | L4600 |
| TnT® SP6 Coupled Reticulocyte Lysate System Trial Size | 8 reactions | L4601 |
| TnT® T7/SP6 Coupled Reticulocyte Lysate System | 40 reactions | L5020 |
| TnT® T7/T3 Coupled Reticulocyte Lysate System | 40 reactions | L5010 |
| TnT® T7 Quick for PCR DNA | 40 reactions | L5540 |

### *E. coli* S30 Extract Systems

| Product | Size | Cat.# |
|---|---|---|
| *E. coli* S30 Extract System for Circular DNA | 30 reactions | L1020 |
| *E. coli* S30 Extract System for Linear Templates | 30 reactions | L1030 |
| *E. coli* T7 S30 Extract System for Circular DNA | 30 reactions | L1130 |

### Rabbit Reticulocyte Lysate Translation Systems

| Product | Size | Cat.# |
|---|---|---|
| Rabbit Reticulocyte Lysate, Nuclease Treated | 30 reactions | L4960 |
| Flexi® Rabbit Reticulocyte Lysate System | 30 reactions | L4540 |

### Rabbit Reticulocyte Lysate/Wheat Germ Extract Combination System

| Product | Size | Cat.# |
|---|---|---|
| Rabbit Reticulocyte Lysate/Wheat Germ Extract Combination System | 24 reactions | L4330 |

## 8. Summary of Change

The following change was made to the 12/17 revision of this document:

1. Section 4.B was updated to include details on instrumentation.