

Catalog Number PL-00N02**Instructions for labeling proteins with terbium for applications involving time resolved fluorescence.****Reagents. Store all reagents at 4°C.**

Reagent TRF. Proprietary chelate (1.46×10^{-6} mol) ~1 mg in a 2ml plastic screw cap vial sealed with parafilm

Reagent 2. Reconstitution solution (2 ml) 2.04 ml 1 M sodium acetate and 0.112 ml 1 M sodium hydroxide in a 2 ml glass vial with red stopper and green crimp

Reagent 3. 1 M $TbCl_3$ (0.2 ml) 2 ml glass vial with grey stopper and silver crimp

Reagent 4. 0.2 M Carbonate buffer pH 8.9 (10 ml) 10 ml glass vial with red stopper and silver crimp

Developing solution (10 ml) 15 ml hdpe screw cap bottle (Catalog Number R-1002-10)

1 Generic conjugation procedure

1.1 Protein preparation. Transfer protein to a suitable solution free of nucleophiles such as amine and sulfhydryl groups. We recommend that the protein conjugation be done in 0.2 M carbonate buffer pH 8.5 to 8.9. If possible, start by dissolving the solid protein in the reaction buffer.

1.2 Reconstitution of proprietary chelate and chelation of terbium. To the container of Reagent 1 add in sequence 78.6 μ l of Reagent 2 and 1.3 μ l (1.33×10^{-6} mol) of Reagent 3. Cap container and vortex approximately 2 minutes until solid dissolves. Let the resulting solution sit for 5 minutes.

1.3 Protein conjugation. At room temperature and with gentle mixing of your protein, add approximately one, two, three or more mols of chelate per mol of protein. Continue mixing for 2 hours.

1.4 Purification. Use a suitable size separation procedure for purification of your protein such as gel filtration or dialysis against a suitable buffer for your protein.

2 Time resolved fluorescence: development and reading

2.1 General instructions. Add 1 ml developing reagent to a terbium free tube. Add less than 50 μ l of sample to developing reagent to achieve desired signal. Mix. Add 25 μ l of 1N hydrochloric acid. Mix. Incubate sample for 1 minute. Add 25 μ l of 1N sodium hydroxide. Mix. Incubate sample for 1 minute. Read sample within 15 minutes. Suggested instrumental parameters: excitation 340 nm, emission 615 nm, total decay time (s) 0.02, delay time (ms) 0.2, gate time (ms) 5.0.

Excitation and emission spectra for terbium obtained using TRDS-Terbium (Catalog Number R-1002-10). Terbium (0.1 mM) in TRDS-Terbium was scanned using a Cary Eclipse. The spectrum on the left is the excitation response using 545nm for emission. The spectrum on the right is the emission response using 297nm for excitation.

