Instructions for Labeling Proteins with Gadolinium

Reagents. Store all reagents at 4°C.

Reagent 1	Proprietary chelate ($7.3x10^{-6}$ mol) ~5 mg in a 2 ml 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 plastic cal vial sealed with parafilm
Reagent 3	1 M $GdCl_3$ (0.2 ml) 2 ml glass vial with gray stoper and silver crimp
Reagent 4	0.2 M carbonate buffer pH 8.9 (10 ml) 10 ml vial with red stopper and silver crimp

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 gadolinium.

To the container of Reagent 1, add in sequence 393 µl of Reagent 2 and 6.66 µl ($6.66x10^{-6}$ 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.

Example conjugation with albumin

2.1 Protein preparation.

Dissolve 50 mg of albumin ($7.3x10^{-7}$ mol) in 10 ml of Reagent 4.

2.2 Reconstitution of proprietary chelate and chelation of gadolinium.

To the container of Reagent 1, add in sequence 393 µl of Reagent 2 and 6.66 µl ($6.66x10^{-6}$ mol) of Reagent 3. Cap container and vortex approximately 2 minutes until solid dissolves. Let the resulting solution sit for 5 minutes.

2.3 Protein conjugation.

At room temperature and with gentle mixing of the albumin, add all of the reconstituted chelate (7.3×10^{-6} mol) to the albumin. Continue mixing for 2 hours.

2.4 Purification.

Dialyze the conjugated albumin against a 10K MWCO membrane. Store at 4°C.

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Instructions for labeling proteins with gadolinium for applications involving magnetic resonance imaging.

Reagents. Store all reagents at 4ºC.

Reagent 1. Proprietary chelate (7.0×10^{-6} mol) ~5 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 $GdCl_3$ (0.2 ml) 2 ml glass vial with grey stopper and green crimp

Reagent 4. 0.2 M carbonate buffer pH 8.5 (10 ml) 10 ml glass vial with red stopper and silver crimp

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. If possible, start by dissolving the solid protein in the reaction buffer Reagent 4. Check pH to be sure it is still 8.5 and adjust as necessary.

1.2 Reconstitution of proprietary chelate and chelation of gadolinium. To the container of Reagent 1 add in sequence 393 ul of Reagent 2 and 6.66 ul (6.66×10^{-6} mol) of Reagent 3. Cap container and vortex approximately 2 minutes until solid dissolves. A short burst of sonication from a bath sonicator can also be used to facilitate dissolution. Let the resulting solution sit for 2 minutes.
This solution is 16.6×10^{-6} mol chelate/ml

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. If possible, monitor the pH and adjust to pH 8.5 as necessary. A small amount of cloudyness may appear at the beginning of the reaction. This is from an initial formation of gadolinium oxide. This will disappear in the course of the reaction.

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. Example conjugation with albumin

2.1 Protein preparation. Dissolve 50 mg of albumin (7.3×10^{-7} mol) in 10 ml of Reagent 4.

2.2 Reconstitution of proprietary chelate and chelation of gadolinium. To the container of Reagent 1 add in sequence 393 ul of Reagent 2 and 6.66 ul (6.66×10^{-6} mol) of Reagent 3. Cap container and vortex approximately 2 minutes until solid dissolves. Let the resulting solution sit for 2 minutes.

2.3 Protein conjugation. At room temperature and with gentle mixing of the albumin, add all of the reconstituted chelate (7.3×10^{-6} mol) to the albumin. Continue mixing for 2 hours.

2.4 Purification. Dialyze the conjugated albumin against a 10K MWCO membrane. Store at 4°C.