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# Protocol for Cell Labeling with CL-30Q02-6 Treated with Polylysine

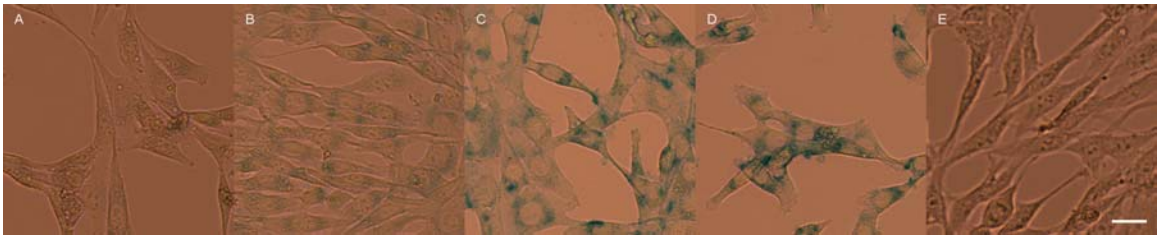


Figure 1. NIH3T3 cells labeled with CL-30Q02-6 / polylysine. Panels A-E were prepared as described in the table below. The concentration of CL-30Q02-6 was 50ug Fe/ml in the cell medium. After incubation with contrast agent cells were fixed and stained with Prussian blue. It is apparent that labeling reaches a maximum in panel C. Bar=20um

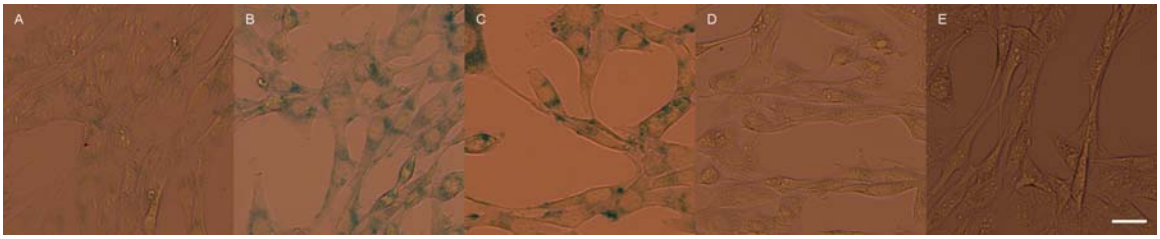


Figure 2. NIH3T3 cells labeled with CL-30Q02-6 / polylysine. Panels A-E were prepared as described in the table below. The concentration of CL-30Q02-6 was 5ug Fe/ml in the cell medium. After incubation with contrast agent cells were fixed and stained with Prussian blue. It is apparent that labeling reaches a maximum in panel B. 5ug Fe /ml. Bar=20um

**Materials:**
Molday ION (10mg Fe/ml), Catalog No. CL-30Q02-6
Poly-L-Lysine (10mg/ml), Catalog No. CL-00-01
Distilled Water

**Procedure:**
1. **Cells** are seeded into a suitable multiwell plate, such as 24-multiwell plate, 1-2 days before the day of the experimental procedure and grown to a sub-confluent state of approximately 70-80% confluence on the day of the experiment.

2. **Pilot experiment of CL-30Q02-6 with polylysine (test-material).** The first study should include a titration of Molday ION, CL-30Q02-6, with varying amounts of poly-L-lysine. By running a pilot study the investigator can select the optimal mixture of CL-30Q02-6 and polylysine for their cell type and experimental conditions. It may be necessary to perform several pilot experiments to determine optimal conditions for your cell type, serum, serum concentration in medium, etc. On the day of the experiment prepare a sufficient amount of the test material by diluting CL-30Q02-6 with distilled water and polylysine as suggested by the table below. Mix the three components and allow to combine for at least one hour. Discard any unused test material after 24 hours. The concentration of the iron in the test materials is approximately 2 mg Fe/ml. Dilute the test materials to a concentration of 100 ugFe/ml in your desired diluent such as medium supplemented with serum. The concentration of the test material is now 2X of the desired concentration needed to be tested.

| CL-30Q02-6 ml | Distilled Water ml | Polylysine ul | Corresponds to panel |
|---|---|---|---|
| | | | |
| 0.1 | 0.4 | 60 | A |
| 0.1 | 0.4 | 30 | B |
| 0.1 | 0.4 | 10 | C |
| 0.1 | 0.4 | 3 | D |
| 0.1 | 0.4 | 0 | E |

After completion of the pilot experiment, the desired ratio of CL-30Q02-6 and polylysine should be used to prepare test reagent in future experiments. Remember to allow the three components to mix for at least one hour and discard after 24 hours.

3. Prior to adding test material, replace the cell medium with fresh medium to ½ the normal working level.

4. Add an equal volume as used in step 3 of freshly prepared 2X test material/medium solution (step 2) to duplicate wells of cells.

5. Place the cells into the incubator for 4 to 20 hours dependent upon the degree of labeling desired. Proceed to cell fixation by treating 4 wells of cells at a time through the fixation process if desired (protocol available from BioPAL). Otherwise remove the medium and replace with fresh culturing medium. (In the case of cell fixation, fresh medium does not need to be added. The cells are washed with PBS with Mg and Ca, and subsequently fixed within several minutes of processing). An example of NIH3T3 cells that have been titrated with CL-30Q02-6 and polylysine per the table above is presented in Figures 1 and 2 which show how both the CL-30Q02-6 and polylysine ratio and the final concentration of iron used to label cells can have a profound effect on extent of cell labeling.

6. If imaging is performed on live cells, allow a suitable time for the cells to recover before further processing or imaging.