

Protocol:	Fluorescent-Tfn pulse and pulse-chase labelling of cells
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1. Step-chase experiment - General protocol

Workflow:

1. Wash cells with CO₂-independent medium twice and put in CO₂-incubator at 37C for 45 min.
2. At 37C water bath, add freshly prepared fluorescently-labeled Tfn solution (10 µg/ml in CO₂-independent medium) at the beginning of every time point
3. Chase required time points
4. When time is over (point 0) put cells immediately on ice and wash once with ICE COLD PBS - (be as fast as possible!)
5. Fix cells with 4% PFA, incubate for 15 min at RT,
6. Wash 3x with PBS
7. Keep at +4C

At this stage, cells can be stained with antibodies or DAPI, if required.

2. Pulse-chase experiment - General protocol

Workflow:

1. Wash cells with CO₂-independent medium twice and put in CO₂-incubator at 37C for 45 min.
2. At 37C water bath, add freshly prepared fluorescently-labeled Tfn solution (10 µg/ml in CO₂-independent medium) at the beginning of every time point
3. Wait for desired time* of pulse and wash cells with CO₂-independent medium
4. Add solution of unlabeled Tfn (100 µg/ml in CO₂-independent medium)
5. Chase required time points
6. When time is over (point 0) put cells immediately on ice and wash once with ICE COLD PBS - (be as fast as possible!)
7. Fix cells with 4% PFA, incubate for 15 min at RT,
8. Wash 3x with PBS
9. Keep at +4C

At this stage, cells can be stained with antibodies or DAPI, if required.

Comments:

- 1) *- time of pulse can be in the range of 0.5 – 10 min.
- 2) If you used coverslips, mount them on microscopic slide. Place inverted coverslip directly onto a clean slide on a drop of mowiol. Avoid excess of mowiol as coverslips may float and

it will take longer to dry. Also avoid air bubbles in mowiol as well as between the coverslip and the slide. Slight pressure with forceps can help contact – but be gentle so as not to crush samples! Place slides in a dark box at 37C, for 45 min or at RT overnight to dry, then clean the surface of the coverslip and use it for fluorescent microscope imaging.

- 3) This protocol can be used for other fluorescent cargos, for example, EGF (50 ng/ml for streptavidin-Alexa conjugates and 10 ng/ml for directly-labeled EGF) or LDL (5-10 μ g/ml).

