Transfection of mRNA LifeAct-TagGFP2 with Fuse-It-mRNA easy

This example experiment describes the transfer of mRNA LifeAct-TagGFP2 into a human cell line (HeLa) using Fuse-It-mRNA easy.

Keywords:
mRNA, LifeAct, F-actin, cytoskeleton, HeLa, GFP, transfection, Fuse-It, membrane fusion, fluorescence microscopy

1. Background

The **mRNA LifeAct-TagGFP2** can be used to visualize F-actin in living cells by fluorescence microscopy in a convenient manner. Here, we combine the LifeAct mRNA technology with a novel membrane fusion-based transfection reagent called **Fuse-It-mRNA easy**.
In the protocol below, the standard protocol given in the "Fuse-It-mRNA easy Instructions" is followed.

2. Material and Equipment Required

For this protocol, the following materials are needed:

Table 1: Material and reagents.

Name	Company	Order No.
Fuse-It-mRNA easy	ibidi GmbH	60505
µ-Dish $^{35\ mm,\ high}$ ibiTreat	ibidi GmbH	81156
mRNA LifeAct-TagGFP2	ibidi GmbH	60151
HeLa cells	DSMZ	ACC-57
RPMI-1640 supplemented with 10% FBS	Sigma-Aldrich Chemie GmbH Sigma-Aldrich Chemie GmbH	R8758 F0804
1x PBS	Sigma-Aldrich Chemie GmbH	D8537

3. Experimental Procedure and Results

Seeding Cells

1. Seed the HeLa cells in the µ-Dish with a density of 10,000 cells/cm².
2. Incubate the cells for 24 hours at 37°C under standard cell culture conditions.

Fusion of HeLa Cells Using Fuse-It-mRNA easy

3. Mix 1 µg of the mRNA LifeAct-TagGFP2 (= 1 µl of 1 µg/µl stock) and 2 µl of the Neutralization Buffer (NB) thoroughly. Incubate for 10 minutes at room temperature.
4. In the meantime, vortex the Fusogenic Solution (FS) until the solution is homogeneous.

5. Add 2.5 µl of FS to the neutralized mRNA (mRNA + NB), then mix thoroughly.
6. Dilute the fusogenic mixture in 1x ice-cold PBS to make a final volume of 250 µl, and mix thoroughly.
7. Remove the culture medium from the cells in the µ-Dish and add 250 µl of the fusogenic mixture drop-wise onto the cells.
8. Incubate for 5 minutes at 37°C.
9. Replace the fusogenic mixture with fresh culture medium to stop the fusion.
10. Incubate the cells for 24 hours at 37°C under standard cell culture conditions.
11. Image the cells by fluorescence and phase contrast microscopy.

Results

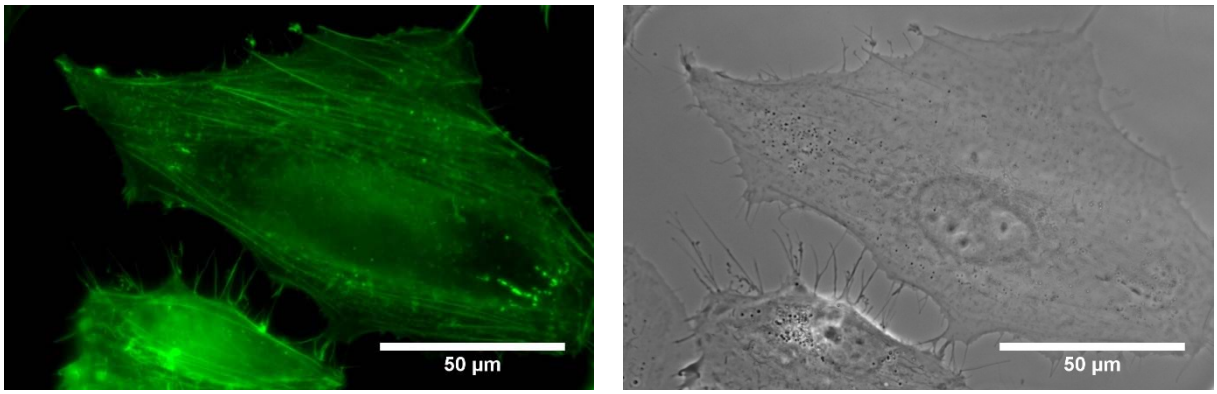

Figure 1: Microscopic images of HeLa cells expressing LifeAct-TagGFP2 24 hours after mRNA transfection. Widefield fluorescence and phase contrast. Objective lens 60x, oil immersion.