

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	Sigma-Aldrich Chemie GmbH	R8758
supplemented with 10% FBS	Sigma-Aldrich Chemie GmbH	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 μ g of the mRNA LifeAct-TagGFP2 (= 1 μ l of 1 μ g/ μ l stock) and 2 μ l 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 μ l of FS to the neutralized mRNA (mRNA + NB), then mix thoroughly.
6. Dilute the fusogenic mixture in 1x PBS to make a final volume of 250 μ l, and mix thoroughly.
7. Remove the culture medium from the cells in the μ -Dish and add 250 μ l 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

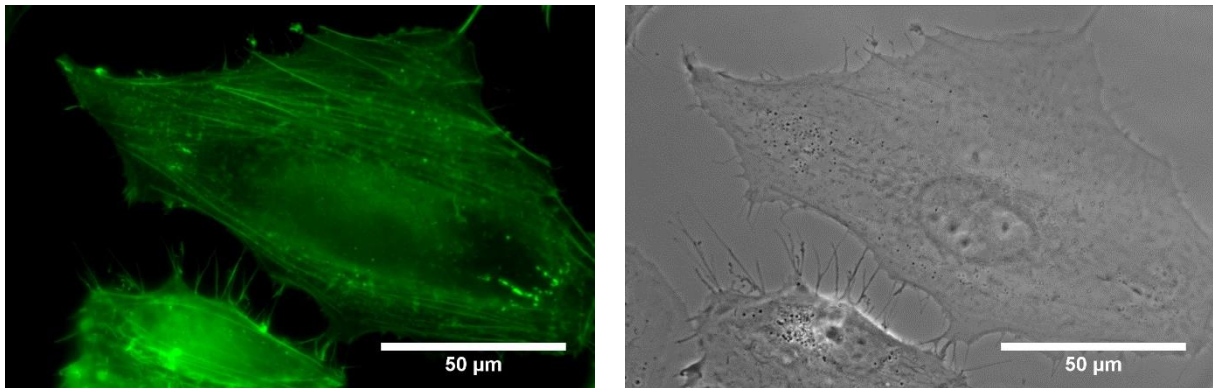


Figure 1: Microscopic images of HeLa cells expressing LifeAct-TagGFP2 24 hours after mRNA transfection. Widefield fluorescence and phase contrast. Objective lens 60x, oil immersion.