ScreenFect®UP-293

For Transfection of Suspension Cells

Transfection Protocol: ScreenFect®UP-293 & ScreenFect®Booster

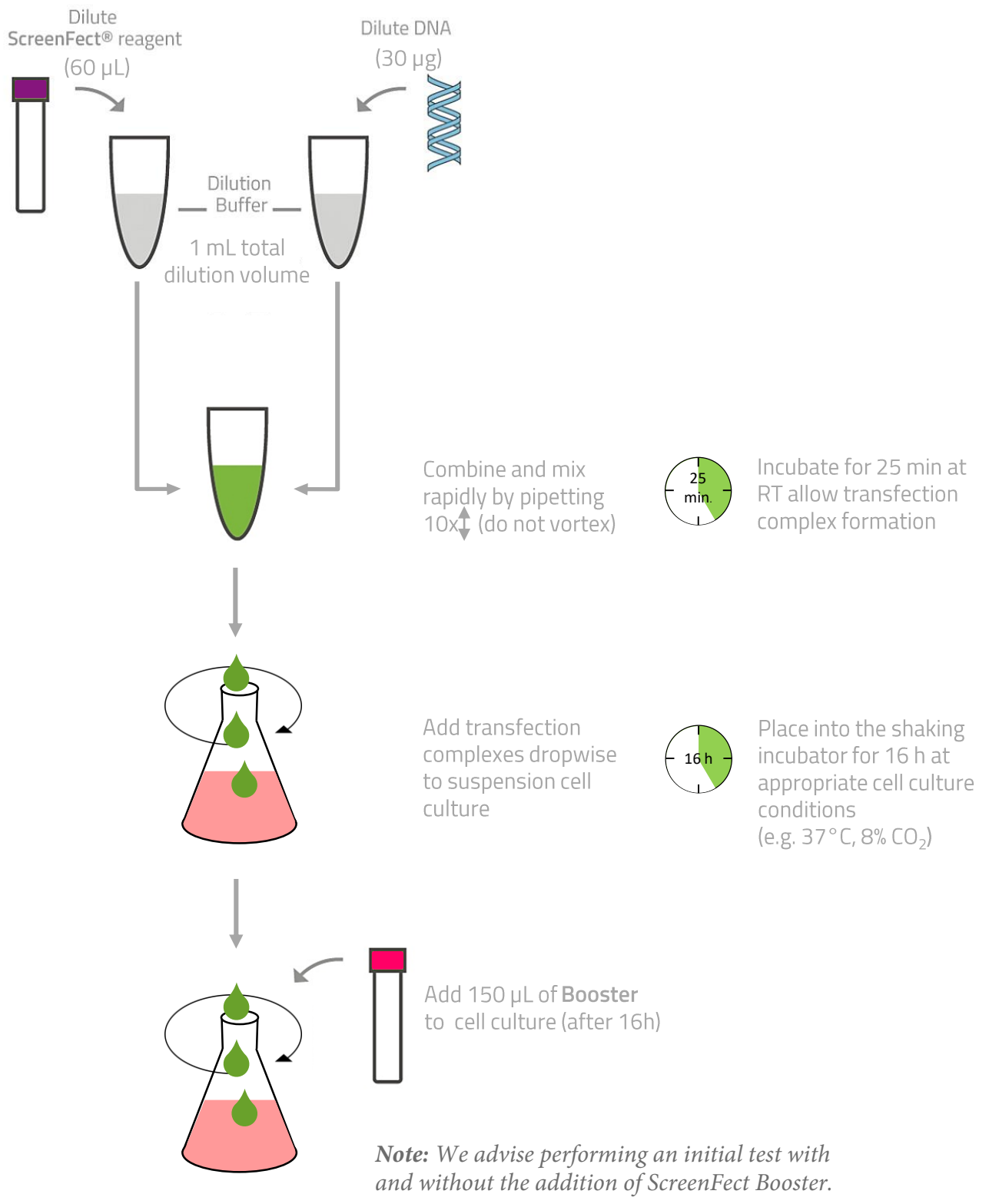
One day prior to transfection

- prepare 30 mL cell cultures at the required cell density (e.g. for HEK293F cells: 0.5–1.5 x 10^6 cells/mL)

On day of transfection

- confirm optimal cell density for transfection (e.g. for HEK 293-F cells: 1.5–2.5 x 10^6 cells/mL, viability ≥ 95 %)
- transfect cells using ScreenFect®UP-293 and add ScreenFect®Booster 16h post-transfection
- follow the protocol shown graphically on the right and described in more detail on the next page

Harvest the cells or media 24–96 h (2-4 days) post transfection, depending on your recombinant protein.



Note: *We advise performing an initial test with and without the addition of ScreenFect Booster.*

ScreenFect® UP-293

For Transfection of Suspension Cells

Transfection Protocol: ScreenFect®UP-293 & ScreenFect®Booster

1 Prepare 30 mL suspension cell cultures in 125 mL Erlenmeyer flasks

- adjust to optimal cell density for transfection
  (e.g. for HEK 293-F cells: 1.5–2.5 x 10^6 cells/mL, viability ≥ 95%)

2 Preparation of reagent and DNA dilutions

- Add 940 µL of Dilution Buffer to a 1.5 mL tube. Pipette 60 µL of ScreenFect®UP-293 directly into the Dilution Buffer and use pipette action to mix and transfer all reagent from tip.
- Next, dilute 30 µg of pDNA in 1 mL of Dilution Buffer (use a 2 mL tube here for the later incorporation of the 1 mL reagent)
- Mix both 1 mL dilutions of reagent/pDNA using brief vortexing or pipette strokes.

3 Initiation of Transfection Complex Formation

- Combine reagent and DNA by pipetting the diluted ScreenFect®UP-293 reagent into the diluted pDNA and immediately mix with 5-10 pipette strokes. We advise against vortexing.
- Incubate for 25 min at RT, without movement, to allow transfection complex (lipoplex) formation.

4 Initiation of Cell Transfection

- Add lipoplexes, in a ***rapid dropwise manner***, to the 30 mL cell cultures, while simultaneously rotating the flask to mix.
- Culture under consistent orbital shaking and appropriate environmental conditions (e.g. 125 rpm in an atmosphere containing 8 % CO2 at 37°C).

5 Booster Addition

- 16 h after transfection, add 150 µL of Booster to the cell cultures, if necessary, and continue incubation.
- We advise testing with/without Booster

***Note**: In some cases the addition of Booster may not be required, or even be detrimental to the final yield of the desired protein. For this reason we always advise performing an initial test with and without the addition of ScreenFect Booster 16 hours post transfection.*

Harvest cells or media 24–96 h post transfection, depending on your recombinant protein. Cells may continue to grow for many days and reach high densities, despite significantly reduced cell viability. If harvesting proteins secreted into the culture medium, remember that fresh medium may be added to the cell pellet (obtained during harvest-1) for continued production in some cases.