ScreenFect® Reagents

For Suspension Cell Transfection

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

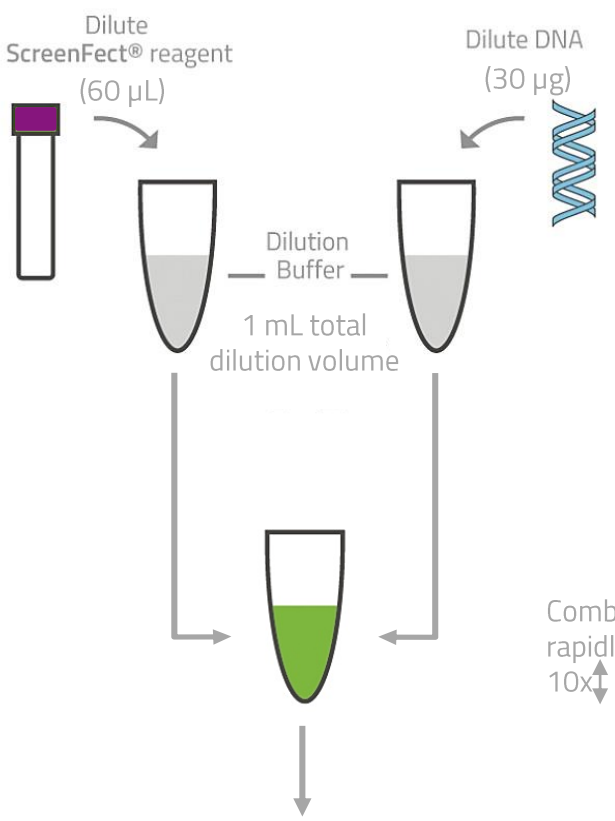
One day prior to transfection

- prepare 30 mL cell cultures at the required cell density (e.g. for HEK 293-F cells: 0.5–1.5 x 10^6 cells/mL)

On day of transfection

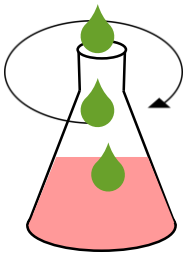
- confirm optimal cell density for transfection (e.g. for HEK 293-F cells: 1.5–2.5 x 10^6 cells/mL, viability ≥ 95 %)
- transfect cells using ScreenFect®UP-293 and add ScreenFect®Booster 16h post-transfection
- follow the protocol (shown on the right and described on the last page)

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

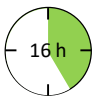


Combine and mix rapidly by pipetting 10x (do not vortex)

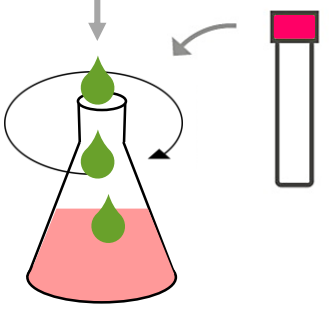
Incubate for 25 min at RT allow transfection complex formation



Add transfection complexes dropwise to suspension cell culture



Place into the shaking incubator for 16 h at appropriate cell culture conditions (e.g. 37 °C, 8% CO_2)



Add 150 µL of **Booster** to cell culture (after 16h)

Reagent	Cat. number	Volume
ScreenFect®UP-293	S-8010-2	0.2 mL
ScreenFect®Booster	S-1003-2	0.5 mL
SFA P-Reagent	S-1002-2	0.1 mL
ScreenFect®Dilution Buffer	S-2001-2	20 mL

ScreenFect® Reagents

For Suspension Cell Transfection

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

1 Preparation of 30 mL cell cultures in 125 mL Erlenmeyer flasks

- adjust cell density to optimal density for transfection
  (e.g. for HEK 293-F cells: 1.5–2.5 x 10^6 cells/mL, viability ≥ 90 %)

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, using same tip, immediately mix with rapid pipette action.
- Next, dilute 30 µg of pDNA in 1 mL of Dilution Buffer. Add the appropriate amount of Dilution Buffer to a 2 mL tube to ensure a final dilution volume of 1mL after adding your pDNA solution.
- Mix both dilutions using brief vortexing or 5 pipette strokes.

3 Initiation of Lipoplex Formation

- Combine reagent and DNA by pipetting the diluted ScreenFect®UP-293 into the diluted pDNA and mix with 5-10 pipette strokes.
- Incubate for 25 min at RT to allow transfection complex (lipoplex) formation

4 Initiation of Cell Transfection

- Add lipoplexes dropwise to the 30 mL cell cultures and immediately place into the incubator.
- 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.
- Continue incubation.

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.

Note: This protocol gives a detailed description on the transfection procedure of ScrenFect®UP-293 when combined with ScreenFect®Booster. If you are testing ScrenFect®UP-293 with P-reagent, you can follow the same steps as described here, but please use the image on the next page of this document and adjust volumes and incubation times accordingly.

ScreenFect® Reagents

For Suspension Cell Transfection

Transfection Protocol: ScreenFect®UP-293 & SFA-P-reagent

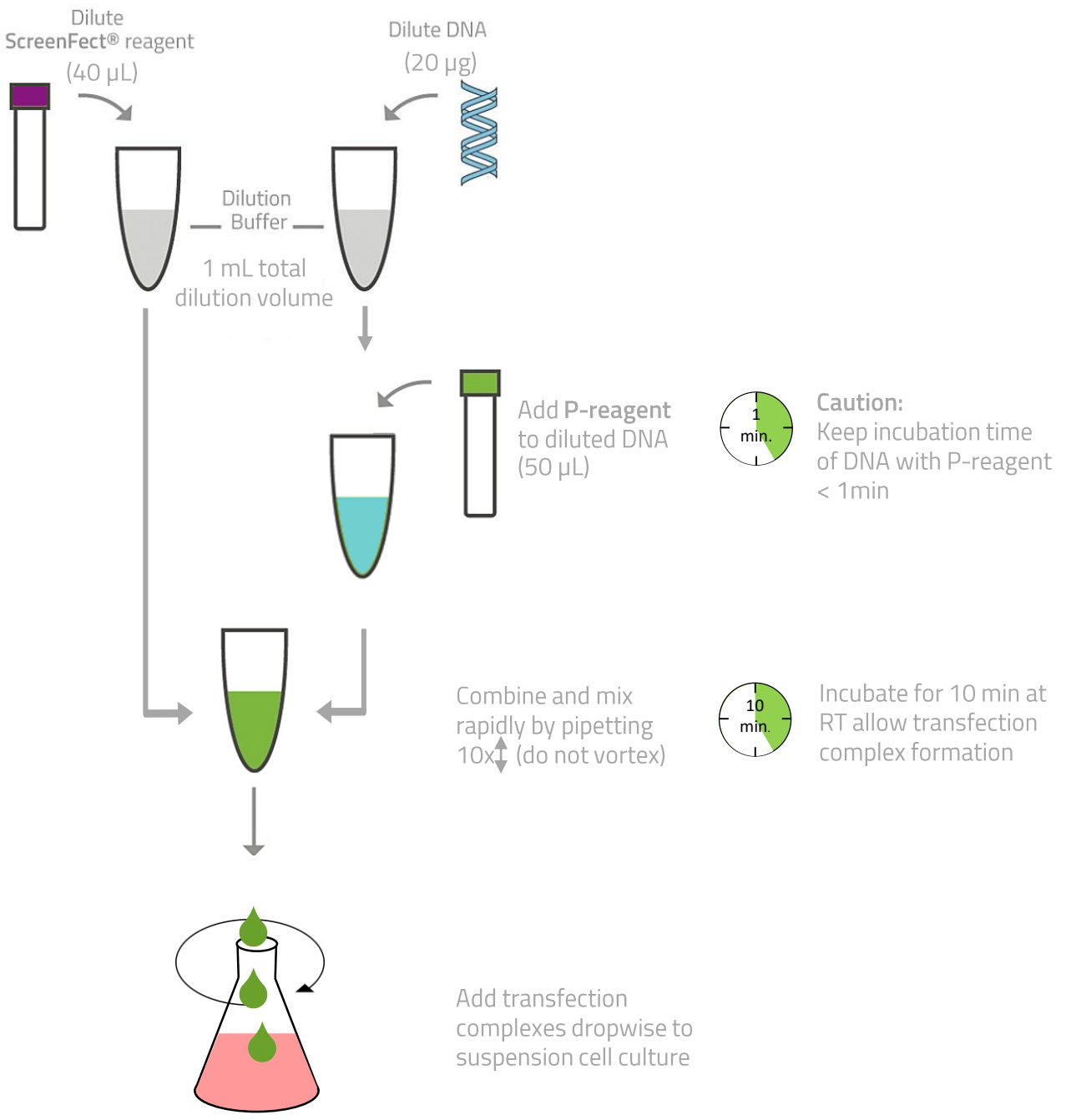
One day prior to transfection

- prepare 30 mL cell cultures at the required cell density (e.g. for HEK 293-F 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 106 cells/mL, viability ≥ 95 %)
- transfect cells using ScreenFect®UP-293, with addition of SFA P-reagent to DNA
- follow the protocol shown here on the right
- if desired, also add ScreenFect®Booster 16h post-transfection

Harvest cells or media 24–96 h post transfection, depending on your recombinant protein



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ScreenFect®Dilution Buffer	S-2001-2	20 mL