Simple Transfection Reagent (Cat: T3080)

Sizes: **500uL (5x 100ul)** *Research use only, not for diagnostic use.*

Storage: **-20°C or -80°C.** • After thawing the solution can be stored at 4 °C for up to 2 months.

Description:

Simple transfection reagent is a ready-to-use polymer-based transfection agent, with high transfection efficiency and lowest toxicity to deliver DNA or siRNA into various primary/ mammalian cell lines.

Workflow Timeline:

Day 1: Seed cells
Day 2:(am/pm) Transfect Cells (am), After 2h-4h transfection - add the complete media (pm).
Day 3:(pm) or more Observe fluorescence, or by FACS analysis.

General Protocol:

Day 1: **Cell Seeding**: Split cells one day before transfection.

a. **6 well plate:** $2x10^5$ cells/well

b. **10 cm dish:** $1x10^6$ cells/dish

The cell density should reach to the 40-90% confluency at the time of transfection.

Day 2: (Am) Prior to transfection, bring Simple-Transfection reagent (T3080) to room temperature and warm the serum-free media (DMEM w/o phenol red) or PBS to 37°C.

1. **Preparation the complex: Transfection agent** (ul) + **DNA**(ug) **(3:1).**
   * Dilute total plasmid DNA (ug)/siRNA in serum-free media (DMEM w/o phenol red) or PBS.
   * Add Simple Transfection agent to the diluted DNA/RNA. (**3:1** ratio of T3050 (ul): DNA (ug))

a. For **6 well plate:** 300ul serum-free media (DMEM)+ **3 ug of DNA**, vortex briefly to mix well, add **9 ul of Simple- Transfection reagent;**

b. For **10cm dish:** 900uL serum-free media (DMEM)+ **9 ug of DNA**, vortex briefly to mix well, add **27ul of Simple-Transfection reagent;**

2. **Immediately pulse vortex for 10 seconds. Spin down briefly.**
3. **Incubate 15 minutes at Room Temperature (RT) for forming the TransFection/DNA or RNA complexes.**(The formation of TransFection/DNA might make solution slightly cloudy).
4. **Aspirate the cell culture medium from each well/dish and add the Transfection/DNA complexes to cells carefully drop-wise.** Gently rock the plate to ensure even distribution. Do not swirl plate and not to dislodge the cells.
5. **Return the well/dish to a 37 °C incubator with 5% CO_2.**

Day 2: (Pm) **Incubate at 37°C for 2h–4h**, add appropriate volumes of complete cell culture medium to the well/ dish and continue to incubate the cells for 12-24 h.
a. For **6 well plate:** add 2 mL complete medium/each well.
b. For **10 cm dish**: add 9 mL complete medium/dish.

Day 3: (Pm) or more: Observe fluorescence, harvest cells, or perform your experiment by FACS analysis.