REAGENT 007

PRODUCT DESCRIPTION:
Reagent 007 is a formulation for transfecting plasmid DNA into eukaryotic serum-free suspension cells

PACKAGE CONTENTS:
Cat# R007-P001, 1mg (powder)
Cat# R007-P010, 10mg (powder)

STORAGE CONDITIONS:
Store at -20°C for up to 1 year.

REQUIRED MATERIALS:
- Plasmid DNA
- Sterile deionized water
- Transfection medium*
- Suitable growth medium
- Microcentrifuge tubes
- V-bottom 96-well plates for DNA:R007 complex formation in 96-well format (optional)
- 15 ml or 50 ml centrifuge tubes for large-scale DNA:R007 complex formation (optional)

*** We have tested and found the following media to be either suitable or unsuitable for use as a transfection medium with Reagent 007:**

SUITABLE
293:
FreeStyle 293 Expression Medium (Gibco, Cat. # 12338)
Pro293s-CDM (Lonza, Cat. #12-025Q)
BalanCDR HEK 293 (Irvine Scientific, Cat. #91165)
Expi293™ (Gibco, Cat. #A1435101)
HEK TF (Xell, cat #861-0001)

CHO:
FreeStyle CHO Expression Medium (Gibco, Cat. #12651)
UltraCHO (Lonza, Cat. # 12-724Q)
PowerCHO-1 (Lonza, Cat. # 12-770Q)
Dynamis™ Medium (Gibco, Cat. #A2661501)
ExpiCHO™ Expression Medium (Gibco, Cat. #A2910001)
CD FortiCHO Medium (Gibco, Cat. #A1148301)
BalanCD Transfectory™ CHO (Irvine Scientific, Cat. #91147)
CHO TF (Xell, cat #886-0001)

UNSUITABLE
QMix1 growth medium
ExCell 302 CHO (Sigma-Aldrich, Cat # 24324C)
PowerCHO-2 (Lonza, Cat # 12-771Q)
CD CHO (Gibco, Cat # 10743-029)
CDM4Mab (GE Healthcare, Cat #SH30802.02)
ActiPro (GE Healthcare, Cat #SH31039.02)
HyCellCHO (GE Healthcare, Cat #SH30949.02)
CDM4CHO (GE Healthcare, Cat #SH30558.02)
CDM4PerMab (GE Healthcare, Cat # SH30871.01)

Supplementation of the transfection medium with additional Pluronic® F-68 or any other anti-clumping agent is not recommended as it may cause a dramatic decrease in transfection efficiency!

PREPARATION OF STOCK AND WORKING SOLUTION
Dissolve lyophilized Reagent 007 in sterile deionized water, 1mg/ml to get 4x stock solution, (do not filtrate, do not freeze, store at +4 degrees up to 1 year). To get working solution dissolve 4x stock solution four times to sterile deionized water (do not filtrate, do not freeze, store at +4 degrees up to 4 months).

PROTOCOL

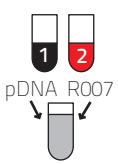
Step 1

1. Seed the cells
- Determine the viable cell density, calculate the volume of cell suspension required to seed a new shake flask or multi-well plate well at a density of 1.5x106 viable cells/ml (see Table below)

- If the media **is suitable** for transfection (list previously): transfer the calculated volume of cells into fresh, pre-warmed and suitable amount of **antibiotic-free medium supplemented with GlutaMAX**. Ideally, the ratio of cells suspension in conditioned medium to added fresh medium for obtaining a density of 1.5 x106 viable cells/ml should be close to 1:1.

- If the media **is not suitable**: collect the cells by centrifugation (~200x g, 5 min) and resuspend in a suitable amount of transfection medium to obtain a density of 1.5 x 106 cells/ml (see Table below)

Step 2



2. Form complexes
- Prepare Reagent 007 Working Solution in an amount sufficient for all transfections. To prepare a Working Solution, dilute the Stock Solution 4 times in deionized water: 1 volume Stock Solution + 3 volumes water. A Working Solution can be stored for further use at +4°C for up to one week.

- Prepare indicated amount of DNA solution in water into appropriate tubes or 96-wells (see recommended amounts and volumes in table)

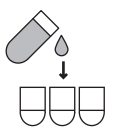
- Add indicated amount (from table) of Reagent 007 Working Solution to the DNA solution and mix immediately by pipetting gently up and down 5-6 times. Do not vortex the complex, it will decrease transfection efficiency!

Step 3

5-10min

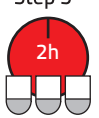
3. Incubate at room temperature for 5 minutes.

Step 4



4. Add DNA/R007 complex by pipetting it slowly to the cells, carefully shaking/rotating the plate or flask at the same time.

Step 5



37c 8%CO_2

5. Incubate at 37°C, in an 8% CO2 environment on an orbital shaker platform for 1.5 hours

Step 6

6. Add growth medium by pipetting it in an amount equal to the transfection medium. The incubation is continued at 37°C, in an 8% CO2 environment on an orbital shaker platform.

IC SAGEN

STANDARD REAGENT VOLUMES

96-well for HTS

Transfection medium	100 µl
Cells	$1.5x10^5$
pDNA in water	0.5 µg in 5 µl
Reagent 007 Working Solution	10 µl
DNA/R007 complex final volume	15 µl
Suitable growth medium	100 µl

6-well

Transfection medium	1ml
Cells	$1.5x10^6$
pDNA in water	1 µg in 30 µl
Reagent 007 Working Solution	20 µl
DNA/R007 complex final volume	50 µl
Suitable growth medium	1 ml

125 ml shaker flask

Transfection medium	10ml
Cells	$1.5x10^7$
pDNA in water	10 µg in 300 µl
Reagent 007 Working Solution	200 µl
DNA/R007 complex final volume	500 µl
Suitable growth medium	10 ml

1 L shaker flask

Transfection medium	100ml
Cells	$1.5x10^8$
pDNA in water	100 µg in 3ml
Reagent 007 Working Solution	2 ml
DNA/R007 complex final volume	5 ml
Suitable growth medium	100 ml

NOTES:
1) For further upscaling just increase all quantities and volumes proportionally
2) For transfection, always use cells in exponential growth phase with a viability >95%
3) If the pDNA is in TE buffer (10 mM TrisHCl, 1 mM EDTA), the volume of TE in the final volume of the complex must not exceed 10%