TransPass™ D2 Transfection Reagent

1-800-632-7799
info@neb.com
www.neb.com

M2554S

0.6 ml **Lot: 0081107**

Store at 4°C **Exp: 7/13**

Description: The TransPass™ D2 Transfection Reagent is a non-lipid cationic polymer designed for transfecting plasmid DNA into many mammalian cell lines, including primary cells and endothelial cell lines. Complex co-transfections of many plasmids can be efficiently transfected using the TransPass D2 Transfection Reagent.

Cell Lines Successfully Transfected: A549, CHO-K1, COS, HCT116, HEK293, HeLa, HepG2, HL60, Jurkat, MCF-7, MDA, NIH3T3 and U20S.

Primary Endothelial cells: HUVEC, HMVEC, lung microvascular endothelial cells, human aortic endothelial cells, bovine aortic endothelial cells.

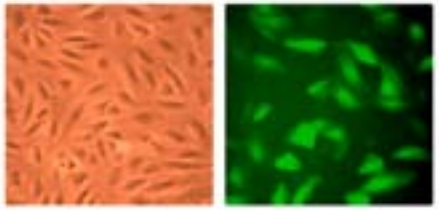

Figure 1: *Human Umbilical Vein Endothelial Cells (HUVEC) transfected with a plasmid expressing GFP using TransPass D2 Transfection Reagent(4).*

Background: The introduction of recombinant DNA into cultured cells or "transfection", has become an essential tool for studying gene function and regulation. Initially, cells were transfected by methods such as Calcium Phosphate co-precipitation, electroporation or viral factors (1, 2). The introduction of cationic lipids or polymers as transfection agents has led to the development of reagents that efficiently deliver DNA through the cell membrane and into the nucleus (3).

Mix well before each use

Quality Control: Each lot of TransPass D2 Transfection Reagent is tested for efficient delivery of two different reporter plasmids expressing luciferase in CHO cells.

Transfection Guidelines: For consistent results, it is important to maintain healthy proliferating cells that are regularly passaged.

It is important that NO heparin and NO antibiotics/antimycotics in the growth medium during transfection.

Use sterile plasmid DNA that is purified by CsCl gradient centrifugation or column chromatography.

Transfection with TransPass D2 Transfection Reagent can be performed in either the presence or absence of serum. Two protocols are given as a guide. The following parameters can be optimized in order to maximize the transfection efficiency for a particular cell line: the cell density at the time of transfection, amount of transfection reagent, amount of plasmid DNA and the culture incubation time before analysis. The specific amount of transfection reagent can be optimized for a particular cell line by performing a simple titration (Figure 2).

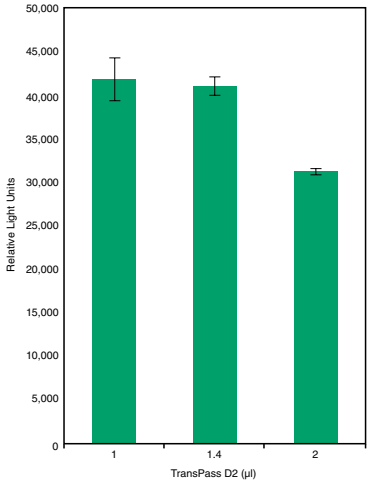

Figure 2: *Titration of TransPass D2 Transfection Reagent in CHO-K1 cells using Protocol 1 and secreted Gaussia luciferase (pCMV-GLuc) as a reporter. Transfections contain 0.7 µg pCMV-GLuc and 250 µl plating medium (10%FBS-F12K) per well. The values obtained from the transfections with the indicated amounts of TransPass D2 Transfection Reagent were obtained from 20 µL of culture medium 24 hr post transfection.*

Protocol 1

Transfection in the presence of serum:

The amounts below are given for a 12-well plate format. Use Table 1 to adjust the reagent volumes for other plate sizes.

1. Plate cells (in complete growth medium containing 5-10% serum and no antibiotics/antimycotics) at an appropriate density so that they will reach 70-80% confluence at the time of transfection.
2. Mix 1.5 µg plasmid DNA in 100 µl **serum-free** medium.
3. Gently mix TransPass D2 Transfection Reagent prior to pipetting. **Do not vortex**. Add 1.5–4 µl to the DNA/medium mix from step 2. Mix gently by flicking the tube.
4. Incubate at room temperature for 20–30 minutes to form the transfection complex.
5. Add the transfection complex mixture to cells. Rock the plate gently in order to evenly disperse the complex mixture.
6. Return the plate to the incubator and incubate 24–72 hours before assaying.
7. Replace medium as needed to maintain healthy cells.

Table 1: Plasmid DNA transfection in the presence of serum

Culture Vessel	Surface (cm^2)	Volume of Plating Medium (per well)	DNA in Serum-free Mixture	TransPass D2 in Transfection
96 well	0.32	75 µl	0.1 µg in 10 µl	0.1–0.3 µl
48 well	0.95	125 µl	0.3 µg in 25 µl	0.3–0.9 µl
24 well	1.9	250 µl	0.7 µg in 50 µl	0.7–2.0 µl
12 well	3.8	500 µl	1.5 µg in 100 µl	1.5–4.0 µl
6 well	9.5	1 ml	3 µg in 250 µl	6–12 µl
60 mm dish	21	2 ml	6 µg in 500 µl	12–20 µl
100 mm dish	55	7 ml	15–20 µg in 1 ml	34–50 µl

(see other side)

Protocol 2

Transfection in the absence of serum:

The amounts below are given for a 12-well plate format. Use Table 2 to adjust the reagent volumes for other plate sizes.

1. Plate cells so they reach a confluence of 60–90% at the time of transfection.
2. For each transfection well, add 1.5 µg of plasmid DNA into 0.6 ml serum-free high glucose DMEM in a microcentrifuge tube.
3. Gently mix TransPass D2 Transfection Reagent tube before pipetting. **Do not vortex.**
4. Add 1.5–3 µl TransPass D2 Transfection Reagent per tube and mix well by flicking the tube. Incubate at room temperature for 20–30 minutes to form the transfection complexes.
5. Wash cells once with serum-free medium.
6. Aspirate the culture medium from the cells and immediately replace with the transfection mixture. Rock the plate gently in order to evenly disperse the complex mixture.
7. Return the plate to the incubator and incubate cells for 2–3 hours.
8. Replace transfection medium with complete growth medium containing serum and incubate for 24–72 hours before assaying.
9. Replace medium 24 hours post-transfection or as needed to maintain healthy cells.

Table 2: Plasmid DNA transfection in the absence of serum

Culture Vessel	Surface (cm^2)	DNA in Serum-free Mixture	TransPass D2 in Transfection
96 well	0.32	0.05–0.5 µg in 10 µl	0.1–0.2 µl
48 well	0.95	0.2–0.5 µg in 25 µl	0.3–0.6 µl
24 well	1.9	0.1–0.6 µg in 0.3 ml	0.5–1.0 µl
12 well	3.8	0.6–2.4 µg in 0.6 ml	1–2 µl
6 well	9.5	1–4 µg in 1 ml	2–4 µl
60 mm dish	21	2–8 µg in 2 ml	4–8 µl
100 mm dish	55	4–10 µg in 4 ml	8–16 µl

Notes On Use:

1. For most cell lines try Protocol 1 first (transfection in the presence of serum). Keep the amount of plasmid DNA constant while varying the amount of TransPass D2 Transfection Reagent. Once the optimal ratio (transfection reagent to DNA) is established, the amount of plasmid DNA and transfection reagent can be increased. Use Table 1 as a guide to optimize the transfection protocol for different plate formats. A convenient method for optimizing transfection conditions is to use pCMV-GLuc Control Plasmid (NEB #N8081), which contains the gene for the secreted *Gaussia* luciferase.
2. For both Protocols 1 and 2, serum-free medium is required to form the transfection complexes (Step 2).
3. Multiple plasmids can be combined (step 2) and simultaneously transfected. For consistent results, the total amount of transfected DNA should stay the same between different wells.
4. If you are using Protocol 2 and the cells are sensitive to prolonged absence of serum, the exposure to the serum-free medium can be minimized by decreasing the incubation time (Step 7).
5. For suspension cells, the following protocol (modification of Protocol 1) is recommended:

   Prepare the plasmid/TransPass D2 complex in 0.5 ml serum-free medium per well of a 12-well plate. Incubate at room temperature for 20–30 minutes. Immediately prior to adding transfection complex (step 5 of Protocol 1), spin down cells to be transfected and resuspend them in high glucose DMEM at a density of 5 x 10^6 cells per ml. Aliquot 100 µl of cell suspension per well of a 12-well plate and add 0.5 ml of the transfection complex. Rock the plate gently in order to evenly disperse the complex mixture and incubate the cells for two hours. Add complete media with serum as follows: 2 ml per well for a 6-well plate, 1 ml per well for a 12-well plate and 0.5 ml per well for a 24-well plate.

References:

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, (2nd ed.). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
2. Ausubel, F. M. et al. (1987) *Current Protocols in Molecular Biology* (2nd ed.). New York: Greene Publishing Associates and Wiley- Interscience.
3. Felgner P.L. et al. (1987) *Proc. Natl. Acad. Sci. USA* 84, 7413–7417.

Companion Products:

pCMV-GLuc Control Plasmid
#N8081S 20 µg

Gaussia Luciferase Assay Kit
#E3300S 100 assays
#E3300L 1,000 assays

TransPass D2 is a proprietary formulation manufactured by Targeting Systems. Please direct any inquiries regarding reagent composition to Targeting Systems.