TransPass™ COS/293 Transfection Reagent

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M2557S 009120614061

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2 x 0.6 ml **Lot: 0091206**

Store at 4°C **Exp: 6/14**

Description: The TransPass™ COS/293 Transfection Reagent is TransPass™ D2 Transfection Reagent, a non-lipid cationic polymer, tested for the transfection of plasmid DNA into COS and HEK293 cell lines in a serum-compatible protocol. Complex co-transfections of many plasmids can be efficiently transfected using the TransPass COS/293 Transfection Reagent.

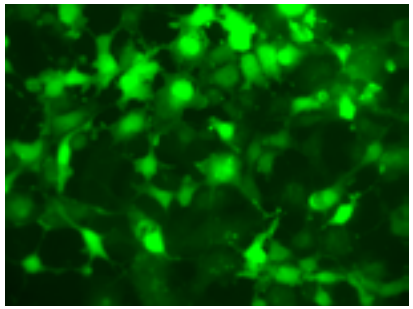

Figure 1: *COS-7 cells grown and transfected on a slide coverslip with a GFP-expressing plasmid and 2 µl TransPass COS/293 Transfection Reagent in 6-well plate format. Data shown is 24 hr post-transfection.*

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 transfection reagent is tested for efficient delivery of two different reporter plasmids in COS-7 and HEK293 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.

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).

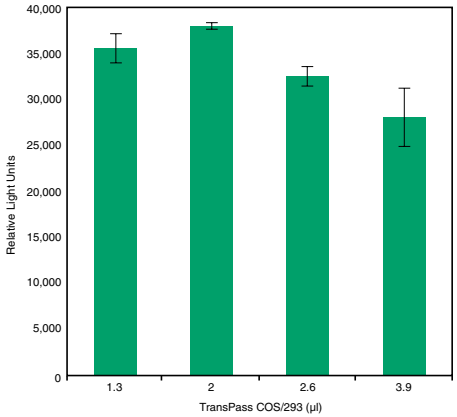

Figure 2: *Titration of TransPass COS/293 Transfection Reagent in HEK293 cells using secreted Gaussia Luciferase (pCMV-GLuc Control Plasmid) as a reporter. Cells are transfected in 12-well plate format and GLuc activity was measured in 10 µl cell supernatant 24 hr post-transfection.*

Transfection Protocol:

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** DMEM medium.
3. Gently mix TransPass COS/293 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 COS/293 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)

Notes On Use:

1. In order to form the transfection complexes (step 2), serum-free medium is required.
2. For optimal transfection of a certain cell density the amount of TransPass COS/293 Transfection Reagent can be titrated while keeping the amount of plasmid DNA constant. Use Table 1 as a guideline for amounts using different plate sizes. 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. See Figure 2 for an example titration in HEK293 cells. In addition, multiple plasmids can be combined (step 2) and simultaneously transfected.

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 COS/293 is a proprietary formulation manufactured by Targeting Systems. Please direct any inquiries regarding reagent composition to Targeting Systems.