## TransPass<sup>™</sup> P Protein Transfection Reagent





# M2563S

 TransPass P: 0.125 ml
 Lot: 0031107
 Exp: 7/13

 Control Protein: 0.02 ml
 Lot: 0051107
 Exp: 7/13

 Store at 4°C

Description: TransPass™ P is a protein transfection reagent that can deliver intact functional proteins and peptides with high efficiency into live mammalian cells via endocytosis. The activity of delivered protein is maintained so active enzymes and other bio-molecules such as antibodies can be directly delivered to cells without the need to obtain and transfect an expression construct.

TransPass P Transfection Reagent forms a noncovalent association with the protein protecting it from degradation upon endocytosis, and thus, allowing the study of protein activity in living cells without the need of permeabilization and fixation.

This protein tranfection reagent has a broad range of cell-type specificity, minimal toxicity, and a high transfection efficiency. A fluorescently labeled control protein (IgG labeled with a 549 fluorophore) is included for optimization of transfection conditions.

**Cell Lines Successfully Transfected:** CHO-K1, COS-7, HeLa, HepG2, Huh-7, HUVEC, IMR-90, MCF-7, MEF, and NIH3T3.

**Storage Note:** TransPass P Transfection Reagent should be stored at 4°C. The fluorescent-labeled control protein (IgG labeled with a 549 fluorophore) should be protected from light and stored at 4°C.

**Quality Control:** Each lot of transfection reagent is tested for efficient delivery of the control protein.

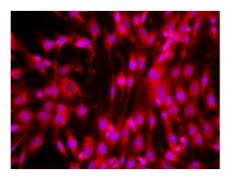


Figure 1: NIH3T3 cells transfected for 2 hours with 6 µl TransPass P Protein Transfection Reagent and 1 µg lgG labeled with a fluorescent dye (a 549 fluorophore). The cells were fixed, DAPI stained and photographed 24 hours post-transfection.

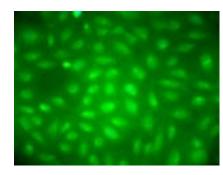
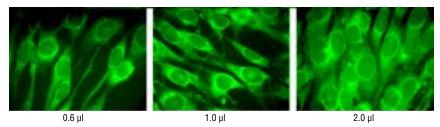


Figure 2: HUVEC cells transfected with an antibody labeled with Oregon Green and TransPass P Transfection Reagent. 1μg of antibody was delivered with 2 μl of TransPass P. The cells were fixed and photographed 24 hours post-transfection.



**Figure 3**: NIH-3T3 cells were transfected with 1  $\mu$ g of a fluorescent peptide (Flu-P1 NEB#P6606) and the indicated amounts of TransPass P Transfection Reagent in a 6-well plate format. The cells were photographed 24 hours post-transfection.

#### **Transfection Protocol:**

The protocol is provided as an example for a 6-well plate format. Amounts for other plate sizes are given in Table 1.

- 1. Set up cells to be transfected so they are approximately  $0.6 \times 10^5$ – $4.0 \times 10^5$  cells per well (approximately 70–80% confluent) at the time of transfection.
- 2. Add 0.5–5 µl of protein to a sterile tube containing the appropriate amount of serum-free medium.
- 3. Add 3 µl of TransPass P Transfection Reagent (mix well before use).
- 4. Gently mix the transfection complex mixture by flicking the tube.
- 5. Incubate at room temperature for 20 minutes.
- Remove serum-containing growth media from cells by aspirating, wash cells with serum-free medium and add 1 ml of serum-free medium to each well.
- 7. Add the transfection complex mixture to cells.
- 8. Return plate to incubator and incubate for 2–5 hours.
- 9. Add 1 ml of complete media (containing 10% serum) to each well.
- Replace media on the following day and continue incubation until assaying. Wash cells with serum-free medium before assaying to remove any untransfected protein.

Table 1: Protein transfection in different plate formats

Culture Vessel	Volume of Plating Medium (per well)	Protein (µg) in serum-free medium	TransPass P in transfection complex mixture*
96 well	100 µl	0.1–0.2 μg in 10 μl	0.2 μΙ
24 well	200 μΙ	0.2–1.0 μg in 50 μl	0.2–2 μl
12 well	0.5 ml	0.5–2.0 μg in 100 μl	0.4–4 μΙ
35 mm dish	1 ml	0.5–5.0 μg in 200 μl	1–10 μΙ
6 well	1 ml	0.5–5.0 μg in 200 μl	1–10 μΙ
60 mm dish	2 ml	2.0-12 μg in 0.5 ml	4–24 μl
10 cm dish	7 ml	6.0-30 µg in 1.0 ml	6–60 µl

The transfection complex mixture is composed of protein and TransPass P Transfection Reagent in serum-free medium. For example, at the incubation step (step 8) (6-well format), transfection complex mixture consisting of 2 µg protein, and 3 µl TransPass P Transfection Reagent in ~200 ul serum-free medium is added to a well containing cells in a 1 ml volume.

#### Notes On Use:

- Use purified proteins and enzymes free of bacteria or toxins.
- The amount of protein and the incubation time with the transfection complex mixture used may be adjusted for optimal transfection efficiency.
- 3. Use 0.5–1  $\mu$ I of the control protein per transfection (6-well plate format).

### **Companion Products:**

Fluorescein-conjugated Chitin Binding Probe #P5211S 100 µI

Rhodamine-conjugated Chitin Binding Probe #P5210S 100  $\mu I$ 

Flu-P1 (lyophilized) #P6606S

0.29 mg

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

(Page 2 M2563)