TransPass™ V

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M2561S 006110713070

M2561S

0.6 ml **Lot: 0061107** **Store at –20°C**

Exp: 7/13

Description: TransPass™ V is an Adenovirus-derived enhancer component that is used along with a transfection reagent. The combination of a TransPass transfection reagent & TransPass-V significantly enhances transfection efficiency in many cell lines and primary cells including endothelial or epithelial (1,2). For example, the combination of TransPass D2 & TransPass V yields optimal plasmid transfection efficiency in suspension cells (Figure 1). This combination has low toxicity and thus, transfected cells show very little, if any, cell death (Figure 2).

*TransPass-V contains a replication-deficient Adenovirus preparation. Because of the nature of this component, it should not be used with cell lines that contain Adenovirus sequences such as HEK-293, to avoid complementation of the virus. Additionally, it is recommended that common laboratory biosafety used in standard Adenovirus work is practiced. For more information see http://oba.od.nih.gov/oba/index.html

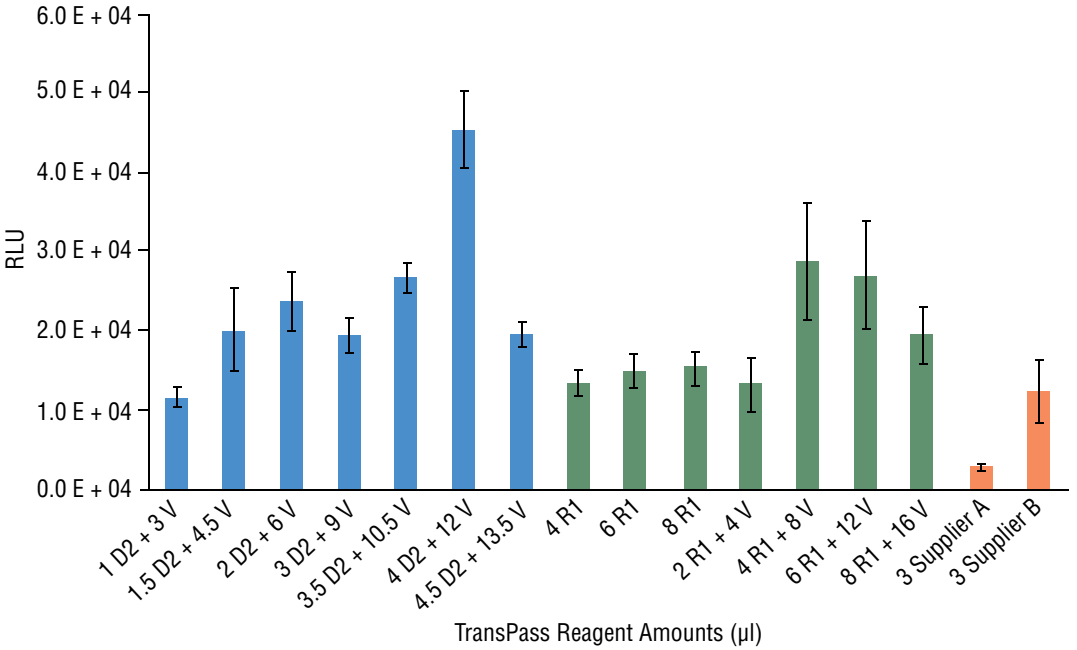

Figure 1: *Transfection efficiency of TransPass reagents in Jurkat. Although Jurkat is a suspension cell line, the proliferating culture of Jurkat, was trypsinized to declump the cells. Trypsin was added to cell pellet followed by 5 minutes of incubation at 37°C, 5% CO_2. The cells were washed once and resuspended in complete growth medium containing no antibiotics/antimycotics before plating at 70% cell density for transfection. Transfection was carried out for 24 hours. Supernatants were collected and assayed for Gaussia Luciferase (GLuc) activity using the Gaussia Luciferase Assay Kit (NEB #E3300).*

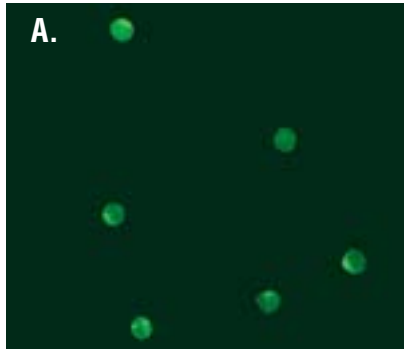

Figure 2: *Transfection of Jurkat cells with Fluorescein-siRNA Transfection Control (NEB #N2100) using a combination of TransPass D2 (NEB #M2554) & TransPass V. Cells were fixed and permeabilized (A) FITC, (B) DAPI.*

Cell Lines Successfully Transfected:

- MEF (TransPass D2 & TransPass V)
- IMR-90 (TransPass D2 & TransPass V)
- HepG2 (TransPass D2 & TransPass V)
- Huh-7 (TransPass D1, TransPass D2 & TransPass V)
- MDCK (TransPass D1, TransPass D2 & TransPass V)
- A549 (TransPass R1 & TransPass V)
- T47D (TransPass R1 & TransPass V)
- Jurkat (TransPass D2, TransPass R1 & TransPass V) (Figure 1)

Quality Control: Each lot of TransPass V is tested along with a TransPass transfection reagent for efficient delivery of a reporter plasmid and/or siRNA in any of the above cell line.

Mix well before each use

Protocol 1:
Combination of TransPass D1 or TransPass D2 & TransPass V

1. In a tube, mix plasmid(s) and/or siRNA in serum free medium.
2. Add TransPass D1 or TransPass D2 to the above mixture.
3. Incubate at room temperature for 25–30 minutes.
4. Add TransPass V to the incubated mixture.
5. (Optional) Incubate at room temperature for 15 minutes.
6. Add the transfection complex mixture to cells (in complete growth media).
7. Incubate at 37°C, 5% CO_2 for 24 hours before replacing media.

Note: Replace media every day, if longer incubation period is required.

Protocol 2:
Combination of TransPass R1 & TransPass V

1. In Tube #1, mix plasmid(s) and/or siRNA together.
2. In Tube #2, add TransPass R1 to serum-free medium.
3. Incubate the content of Tube #2 at room temperature for 15 minutes.
4. Add the content of Tube #1 to Tube #2.
5. Incubate at room temperature for 15 minutes.
6. Add the transfection complex mixture to cells (in complete growth media).
7. Incubate at 37°C, 5% CO_2 for 24 hours before replacing media.

Note: Replace media every day, if longer incubation period is required.

Table 1: *Transfection complex mixtures using TransPass D1 or TransPass D2 & TransPass V*

Culture Vessel	Surface Area	Volume of Plating Medium (per well)	*Total DNA in Serum-free Medium Volume (per well)	TransPass D1 or D2 (per well)	TransPass V (per well)
96 well	0.32 cm^2	100 µl	0.1 µg in 10 µl	0.1–0.3 µl	0.1–0.9 µl
48 well	0.95 cm^2	250 µl	0.3 µg in 25 µl	0.3–0.9 µl	0.3–2.7 µl
24 well	1.9 cm^2	500 µl	0.7 µg in 50 µl	0.7–2.1 µl	0.7–6.3 µl
12 well	3.8 cm^2	1 ml	1.5 µg in 150 µl	1.5–4.5 µl	1.5–13.5 µl
35 mM or 6 well	9.5 cm^2	2 ml	3 µg in 250 µl	3–9 µl	3–27 µl
60 mM dish	21 cm^2	5 ml	6 µg in 500 ml	6–18 µl	6–54 µl
100 mM dish	55 cm^2	15 ml	18 µg in 1 ml	18–54 µl	18–162 µl

Table 2: *Transfection complex mixtures using TransPass R1 & TransPass V*

Culture Vessel	Surface Area	Volume of Plating Medium (per well)	*Total DNA in Serum-free Medium Volume (per well)	TransPass R1 (per well)	TransPass V (per well)
96 well	0.32 cm^2	100 µl	0.1 µg in 10 µl	0.3–0.5 µl	0.3–1.5 µl
48 well	0.95 cm^2	250 µl	0.3 µg in 25 µl	1–1.5 µl	1–4.5 µl
24 well	1.9 cm^2	500 µl	0.7 µg in 50 µl	2–3 µl	2–9 µl
12 well	3.8 cm^2	1 ml	1.5 µg in 150 µl	4–5 µl	4–15 µl
35 mM or 6 well	9.5 cm^2	2 ml	3 µg in 250 µl	10–12.5 µl	10–37.5 µl
60 mM dish	21 cm^2	5 ml	6 µg in 500 ml	20–28 µl	20–84 µl
100 mM dish	55 cm^2	15 ml	18 µg in 1 ml	52–73 µl	52–219 µl

**For plasmid and siRNA transfection, we recommend 20–100 nM siRNA per well in addition to the suggested DNA amount and the 1:1, 1:2 or 1:3 ratio (TransPass transfection reagent: TransPass V).*

Notes On Use:

1. Transfection complex mixture must be made in serum-free media.
2. For a transfection complex mixture containing plasmid(s) and/or siRNA, the plasmids and/or siRNA should be mixed well together before adding them to the serum-free media, followed by the TransPass reagents.
3. The mixture of plasmid(s) and/or siRNA & a TransPass transfection reagent in serum-free media should be incubated at room temperature for 25–30 minutes before adding TransPass V.
4. Recommended ratios of TransPass transfection reagent & TransPass V are 1:1, 1:2 & 1:3.
5. For transfection, it is important that cells are resuspended & plated out in complete growth media containing no antibiotics/antimycotics.
6. It is important that transfection is carried out in non-coated vessels (plates, flasks, etc.).

References:

1 Raja-Walia, R. et al. 1995) *Gene Ther.* 2, 521–530.
2. Stecenko, A. et al. (2000) *Exp. Lung Res.* 26,179–201.

Companion Products:

TransPass R1 Transfection Reagent
#M2551S 0.4 ml

TransPass D1 Transfection Reagent
#M2553S 0.6 ml
#M2553L 3.0 ml

TransPass D2 Transfection Reagent
#M2554S 0.6 ml
#M2554L 3.0 ml

pCMV-GLuc Control Plasmid
#N8081S 20 µg

pGLuc-Basic Vector
#N8082S 20 µg

pTK-GLuc Vector
#N8084S 20 µg

pGLuc Mini-TK Vector
#N8086S 20 µg

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

LumiFlex™ GLuc Assay Kit
#E3308S 100 assays
#E3308L 1,000 assays

Luciferase Cell Lysis Buffer
#B3321S 25.0 ml

TransPass V is a proprietary formulation manufactured by Targeting Systems. Please direct all inquirires regarding reagent composition to Targeting Systems.