TransPass™ HUVEC Transfection Reagent

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M2558S 006110713070

M2558S

TransPass-V: 2 x 0.6 ml	**Lot: 0061107**	**Store at -20°C**
HUVEC Reagent Component: 0.6 ml	**Lot: 0061107**	**Store at 4°C Exp: 7/13**

Description: The TransPass™ HUVEC Transfection Reagent is designed specifically for transfecting endothelial cells including HUVEC, HMVEC, human aortic endothelial cells, etc. with optimal transfection efficiency (Figure 1). TransPass HUVEC Transfection Reagent consists of two components: the HUVEC Reagent Component, a non-lipid cationic transfection reagent, and TransPass-V*, an Adenovirus-derived component.

The addition of TransPass-V significantly enhances lipid-mediated transfection efficiency in many cell lines and primary cells including endothelial or epithelial (1,2).

The combination with the HUVEC Reagent Component yields optimal plasmid DNA transfection efficiency in HUVEC and many endothelial cell lines (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 should be practiced. For more information see http://oba.od.nih.gov/oba/index.html

Cell Lines Successfully Transfected:

- HUVEC cells (Human umbilical vein endothelial cells (70–90%)
- Dermal microvascular endothelial cells (40%)
- Lung microvascular endothelial cells (70–90%)
- Human aortic endothelial cells (50%)
- Bovine aortic endothelial cells (60–70%)
- Rat endothelial cells (60–70%)

Mix Well Before Each Use

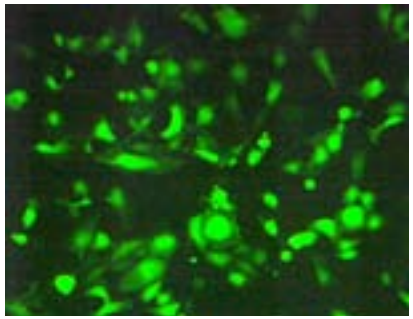

Figure 1: *Transfection of primary Human Umbilical Vein Endothelial Cells (HUVEC) with a GFP-expressing vector using TransPass HUVEC Transfection Reagent. A transfection complex mixture composed of 6 µg of GFP expressing plasmid, 12 µl of HUVEC Reagent Component and 25 µl TransPass-V in 0.5 ml of high glucose media was added to a 60-mm dish of HUVEC in freshly supplmented EBM containing 10% fetal calf serum, followed by an overnight incubation. Media was replaced with complete growth media on the following day. Data courtesy of Dr. Michael Potente, Department of Cardiology, University of Frankfurt, Germany.*

Quality Control: Each lot of transfection reagent is tested for efficient delivery of a reporter plasmid in HUVEC.

Guidelines for Endothelial Cell Culture:

- Use early passages of cells (up to the 6th passage) for transfections.
- Use non-collagen and non-gelatin coated tissue culture plates only (transfection of cells plated on collagen-coated surfaces may result in lower transfection efficiency).
- Recommended media: MCDB131 from VEC technologies, Media 199 plus supplements and 20% fetal calf serum or EBM from Cambrex plus supplements and 10% fetal calf serum. Cells grown in these media appear healthier and give higher transfection efficiency.
- Clonetics HUVEC cells have given very good transfection results.

Transfection Guidelines:

- For consistent results, it is important to maintain healthy proliferating cells that are regularly passaged.
- If cells have been grown in medium containing heparin, they must be washed after trypsinizing and resuspended in growth medium without heparin and antibiotics/antimycotics before plating for transfection.
- It is important that NO heparin and NO antibiotics/antimycotics are present in the growth medium during transfection.
- Use sterile plasmid DNA purified by CsCl gradient centrifugation or column chromatography.
- The amount of plasmid DNA per transfection can be varied, and the ratio of HUVEC Reagent Component to TransPass-V should be kept between (1:1) and (1:2).

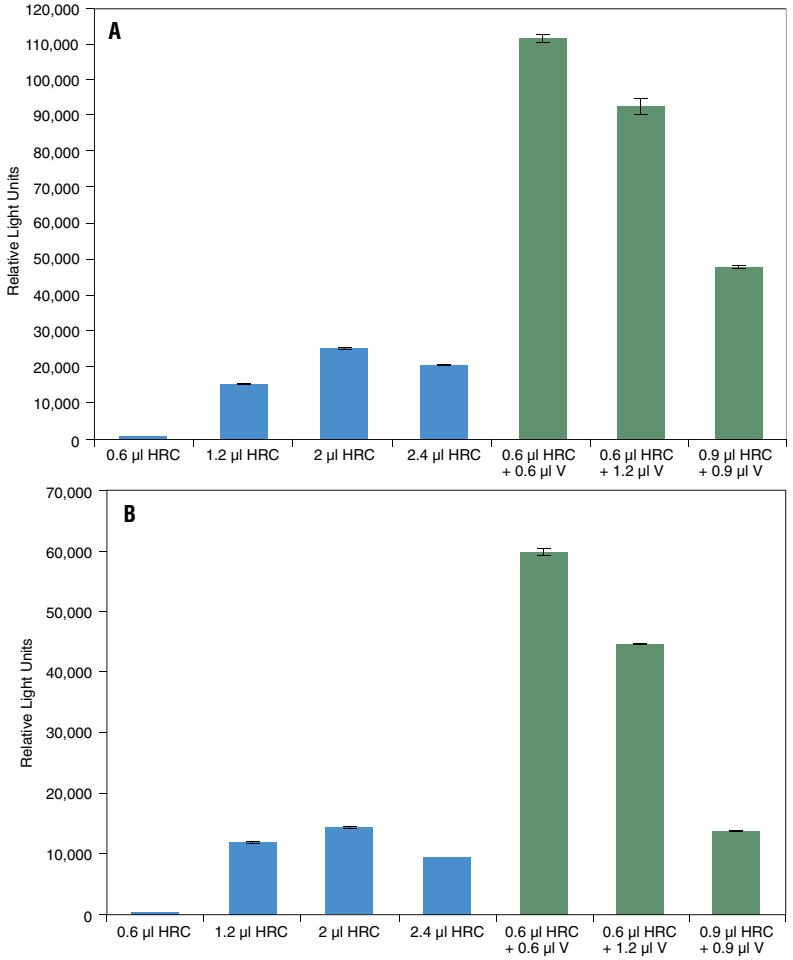

Figure 2: *Pooled HUVEC (Cambrex, C2519A)(A) or HUVEC (Cambrex, C2517A) (B) were transfected with pCMV-GLuc Control Plasmid (NEB #N8081), a plasmid expressing secreted Gaussia luciferase, using either HUVEC Reagent Component (HRC) alone or a combination of HRC and TransPass-V. Briefly, cells were transfected in 24-well plate format (approximately 80% cell density) with 0.6 µg pCMV-GLuc in the presence of serum. After 24 hours of transfection, GLuc activity was assayed from the cell supernatant.*

(see other side)

CERTIFICATE OF ANALYSIS

Transfection Protocol:

Use Table 1 to select the reagent volumes for various plate sizes.

1. Plate cells at an appropriate density so they will reach 70–80% confluence at the time of transfection (plating media should contain 10–20% serum).
2. Mix plasmid DNA with **serum-free** DMEM (Table 1).
3. Add the appropriate volume of HUVEC Reagent Component.
4. Add the appropriate volume of TransPass V.
5. Gently mix the transfection complex mixture by flicking the tube.
6. Incubate at room temperature for 20–30 minutes.
7. Add the transfection complex mixture to cells (Do not remove the serum-containing medium). Rock the plate gently in order to evenly disperse the transfection complex mixture.
8. Return the plate to the incubator and incubate 24–48 hours before assay.

Note: The recommended ratio of HUVEC Reagent Component to TransPass V is between 1:1 and 1:2.

Table 1: DNA Transfection using TransPass HUVEC Transfection Reagent in different plate sizes

Culture Vessel	Volume of Plating Medium (per well)	DNA in serum-free medium volume	HUVEC Reagent component in transfection complex mixture*	TransPass V in transfection complex mixture*
96 well	100 µl	0.2 µg in 25 µl	0.1–0.2 µl	0.1–0.4 µl
24 well	500 µl	0.8 µg in 50 µl	0.5–1 µl	0.5–2 µl
12 well	1 ml	1.6 µg in 150 µl	1–2 µl	1–4 µl
35 mm dish	2 ml	3 µg in 250 µl	4–6 µl	4–12 µl
6 well	2 ml	3 µg in 250 µl	4–6 µl	4–12 µl
60 mm dish	5 ml	6 µg in 0.5 ml	8–12 µl	8–24 µl
100 mm dish	15 ml	18 µg in 1.5 ml	16–25 µl	16–50µl

* *The transfection complex mixture is composed of plasmid DNA and TransPass components in serum-free medium. For example, for a 12-well format, transfection complex mixture consisting of 1.6 µg plasmid, 1 µl HUVEC Reagent Component, 1 µl TransPass V and 150 µl serum-free medium is added to a well containing cells in 1 ml of 10–20% serum containing medium.*

Notes On Use:

1. Do not vortex HUVEC Reagent Component; just gently flick the tube to mix before use.
2. TransPass V must be thawed completely on ice and vortex before each use.
3. We highly recommend that the growth medium be changed 24 hours after transfection.

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:

pCMV-GLuc Control Plasmid
#N8081S 20 µg

pGLuc Basic Vector
#N8082S 20 µg

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

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

Fluorescein-siRNA Transfection Control
#N2100S 1 nmol

TransPass HUVEC and TransPass V are proprietary formulations manufactured by Targeting Systems. Please direct all inquiries regarding reagent compositions to Targeting Systems.