TransPass™ R2 Transfection Reagent

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M2552S 007110813080

M2552S

Solution A: 0.5 ml **Lot: 0071108** **Exp: 8/13**
Solution B: 1.0 ml **Store at 4°C**

Description: The TransPass™ R2 Transfection Reagent is a two component non-liposomal reagent combination developed specifically for efficient transfection of siRNA in a variety of mammalian cell lines that include "difficult to transfect" cells such as primary cells, endothelial cells, lymphocytes, muscle cells, etc. It shows reduced levels of cell toxicity as compared to cationic liposome-based reagents.

NEB has determined that in addition to synthetic siRNAs, siRNA mixtures made with ShortCut® RNase III (NEB #M0245) can be very efficiently transfected in many mammalian cell lines with the TransPass™ R2.

Efficient delivery of labeled siRNA and silencing of endogenous genes has been achieved in many cell lines including:

A549, COS-7, HEK293, HepG2, NIH3T3, HeLa, OVCAR-3, HUVEC, HMVEC, THP-1, B-lymphoma cells, vascular smooth muscle cells, human lens epithelial cells.

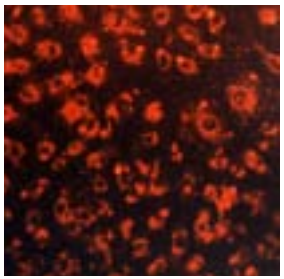

Figure 1: *OVCAR cells transfected with a Cy3-labeled siRNA using TransPass R2.*

Note: Store at 4°C in tightly capped tubes wrapped in parafilm. Do not freeze. Mix well before each use.

Background: RNA Interference (RNAi) is a method of post-transcriptional gene silencing in which the introduction into an organism of double-stranded RNA corresponding to a transcribed sequence results in degradation of the corresponding mRNA (for reviews, see references 1–8). Most mammalian cells treated with long dsRNA (over 30 bp) respond by a non-specific suppression of gene expression as well as apoptosis via the interferon response pathway (6).

In order to perform RNAi in mammalian cells, short dsRNAs (21–23 bp) are currently used because they are able to bypass this general non-specific response and achieve gene target-specific silencing via RNAi (5–7). To this end, synthetic oligo-ribonucleotides with 2 base 3´-OH overhangs (short interfering RNAs, siRNAs) are often used. One shortcoming of this approach is the difficulty of choosing effective 21 bp sites on the target RNA since the silencing effectiveness of siRNAs is highly dependent on the location of the corresponding target site (8).

Another approach, which mimics siRNA production *in vivo* by Dicer endonuclease (9), is to digest large dsRNA *in vitro* with ShortCut RNase III (see ShortCut RNAi kit Manual, NEB #E2450) which results in complete conversion of large dsRNA into a size optimal for RNAi (18–25 bp)(10).

Quality Controls:

Each lot of TransPass R2 Transfection Reagent is tested by NEB for efficient delivery of labeled synthetic siRNA into different cell lines and for endogenous gene silencing using ShortCut siRNA Mixes (Figures 1,2).

siRNA Transfection Guidelines: The transfection of siRNA must be optimal in order to obtain maximum silencing of a target gene. Optimizing the following parameters may be necessary in order to maximize the transfection efficiency for a particular cell line: cell density at the time of transfection, amount of transfection reagent, amount of siRNA and culture incubation time before analysis.

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.

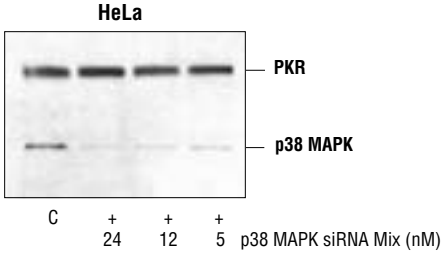

Figure 2: *HeLa cells were transfected with different concentrations of p38 MAPK ShortCut siRNA Mix (+) or 24 nM eGFP ShortCut siRNA Mix (C) using TransPass R2 Transfection Reagent. Effective silencing of p38 MAPK is achieved at low siRNA nanomolar concentration 48 hours post-transfection. p38 MAPK was detected in a Western blot of cell lysates using Cell Signaling Technology (CST #9217) Antibody and PKR loading control was detected using (CST #3072) Antibody.*

siRNA Transfection Protocol:

The following protocol is given with amounts for a 12-well plate format. Use **Table I** to adjust the reagent volumes for other size plates.

1. Plate cells in complete growth medium containing 10% serum and no antibiotics/antimycotics at an appropriate density, so that they will reach 40-60% cell density at time of transfection.
2. Add 1.25 µl TransPass R2 Transfection Solution A to 0.6 ml serum free medium (DMEM high glucose medium) and mix thoroughly, add 2.5 µl TransPass R2 Transfecion Reagent Solution B and mix thoroughly.
3. Add 0.1-1 µl siRNA[1] to the diluted transfection reagent mix gently the tube and incubate for 20 minutes at room temperature to form the transfection complexes.
4. Wash cells once with serum-free medium.
5. Aspirate the culture medium from the cells and immediately replace with the transfection mixture. Evenly disperse the siRNA complexes by gently rocking the plate.
6. Incubate the cells for 2–4 hours. Add 1 ml of complete medium containing serum per well.
7. Return the plate to the incubator and incubate cells overnight.
8. Replace the transfection medium with complete medium containing serum and incubate 24-72 hours before assaying target gene inhibition.

[1] For a stock of siRNA at 10 µM, the final concentration of siRNA will be 2.5–25 nM.

Typical cell incubation time points for detecting target gene inhibition are 24–48 hours for mRNA and 48–72 hours for protein.

Plate Size	6	12	24	96
High Glucose DMEM	1200 µl	600 µl	400 µl	125 µl
TransPass R2 Solution A	2.5 µl	1.25 µl	1 µl	0.3 µl
TransPass R2 Solution B	5 µl	2.5 µl	2 µl	0.6 µl
siRNA (10 µM)	0.3–3 µl	0.15–1.5 µl	0.1–1 µl	0.03–0.3 µl
Final Volume	1.2 ml	0.6 ml	0.4 ml	0.125 ml

Table I. siRNA Transfection

Volumes are shown for one transfection per well for the indicated size plates. For the listed volumes shown, the range of siRNA concentration will be 2.5–25 nM.

Notes:

1. It is important to maintain healthy cells. Some cell lines become more sensitive to transfection agents after a large number of passages. It is advisable to use cells subjected to a similar number of passages to ensure reproducible transfection results in different experiments.
2. It is recommended that control transfections be performed by varying cell confluence (40–90%). In general, low cell density or too much transfection reagent increase the risk of cell toxicity. The transfer medium may be replaced 2 hours after transfection with fresh complete medium to increase cell viability.
3. In order to easily estimate the efficiency of transfection of particular cell lines use Fluorescein-siRNA Transfection Control (NEB #N2100).
4. TransPass R2 reagent has been developed for the transfection of siRNA, siRNA mixtures, *in vitro* transcribed RNA hairpins etc. It has not been optimized for plasmid DNA transfection. The transfection efficiency of plasmic DNA varies from cell line to cell line. For efficient DNA transfection we recommend the use of one of the DNA transfection reagents: TransPass D1 (NEB #M2553) or TransPass D2 (NEB #M2554).
5. TransPass R2 Transfection Reagent can be safely stored for 6 months at 4°C.

(see other side)

CERTIFICATE OF ANALYSIS

References:

1. Fire, A. et al. (1998) *Nature* 391, 806–811.
2. Bass, B.L. (2000) *Cell* 101, 235–238.
3. McManus, M.T. and Sharp, P.A. (2002) *Nature Reviews, Genetics* 3, 737–747.
4. Stark et al. (1998) *Ann. Rev. Biochem* 67, 227–264.
5. Elbashir, S.M. et al. (2001) *Nature* 411, 494–498.
6. Sharp, P.A. and Zamore, P.D. (2000) *Science* 287, 2431–2433.
7. Brummelkamp, T.R. et al. (2002) *Science* 296, 550–553.
8. Holen, T. et al. (2002) *Nucleic Acids Res.* 30, 1757–1766.
9. Bernstein, E. et al. (2001) *Nature* 409, 363–366.
10. Xiao, J. et al. (2009) *RNA* 15, 984–991.

TransPass R2 is a proprietary formulation manufactured by Targeting Systems. Please direct any inquiries regarding reagent composition to Targeting Systems.