

DELIVERX AND DELIVERX PLUS siRNA TRANSFECTION QUICK REFERENCE GUIDE

IMPORTANT If using the DeliverX or DeliverX Plus siRNA transfection reagents for the first time, please refer to the DeliverX and DeliverX Plus Transfection Kits User Manual to review safety precautions and detailed procedures.

Note For assay optimization, we recommend that you perform a range of titers for cell densities and siRNA concentrations.

Cell Preparation Guideline

Parameter	6-well	12-well	24-well	96-well
Adherent Cells Grow cells to 30–70% confluence. Use cells from passages 4–20.				
Seeding density/well	150-300 × 10 ³	50-200 × 10 ³	25-75 × 10 ³	5-10 × 10 ³
Seeding volume/well	2 mL	1 mL	500 µL	100 µL
Suspension Cells Grow cells to 40–80% confluence or to mid-log phase. Use cells from passages 4–20.				

Prepare Transfection Complex

The following procedure provides volumes for preparing 1X, 2X, and 3X scale working transfection complexes. To prepare larger volumes of working transfection complexes, prepare multiple replicate working transfection complexes in parallel and then combine them into a single tube to produce bulk volumes of working transfection complexes.

Step	Action	Scale-Up Volumes and Sonication Times		
		1X	2X	3X
1	Prepare 5 µM siRNA working stocks using TE buffer.			
2	Dilute 5 µM siRNA working stocks with Buffer-1. a. Add Buffer-1: b. siRNA:	37 µL 13 µL	74 µL 26 µL	111 µL 39 µL
3	Prepare siRNA Transfection Reagent. a. Sonicate the siRNA Transfection Reagent: b. Mix Buffer-2 with: c. siRNA Transfection Reagent: d. Vortex and sonicate solution again for:	3–5 min 42 µL 8 µL 3–5 min	3–5 min 84 µL 16 µL 3–5 min	3–5 min 126 µL 24 µL 3–5 min
4	Form concentrated siRNA transfection complex: a. Combine appropriate tubes from steps 2 and 3. b. Briefly vortex and then incubate for 20 min at 37 °C.			
5	Prepare complex dilution buffer. a. Mix Buffer-1 with: b. Buffer-2:	200 µL 200 µL	400 µL 400 µL	500 µL 500 µL
6	Prepare working siRNA transfection complex: a. Add complex dilution buffer to prepared concentrated siRNA transfection complexes: Note The working siRNA transfection complex is ready for transfection (yielding 30 nM siRNA delivery concentration) or further dilution with the complex dilution buffer.	300 µL	600 µL	900 µL

Transfecting Cells

Adherent Cells

Step	Action	6-well	12-well	24-well	96-well
1	Wash cells with 1X PBS.	1,500 μ L/well	750 μ L/well	400 μ L/well	100 μ L/well
2	Add working transfection complex.	300 μ L/well	200 μ L/well	150 μ L/well	30 μ L/well
3	Incubate at room temperature for:	3–5 min	3–5 min	3–5 min	3–5 min
4	Add serum-free media. (You can use serum-containing media but not all cell types will respond in the presence of serum).	300 μ L/well	200 μ L/well	150 μ L/well	30 μ L/well
5	Incubate under normal cell culture conditions for:	2–4 hr	2–4 hr	2–4 hr	2–4 hr
6	Add complete growth media.	1,000 μ L/well	670 μ L/well	500 μ L/well	100 μ L/well
7	Incubate under normal cell culture conditions for:	24–72 hr	24–72 hr	24–72 hr	24–72 hr
8	Quantify mRNA and/or protein knockdown.				

Suspension Cells (Reverse Transfection)

Step	Action	6-well	12-well	24-well	96-well
1	Wash suspension or trypsinized adherent cells with 5 mL of 1X PBS.				
2	Resuspend cells in 1X PBS to a concentration of:	0.3–0.9 $\times 10^6$ cells/mL	0.1–0.4 $\times 10^6$ cells/mL	5 $\times 10^6$ cells/mL	1 $\times 10^6$ cells/mL
3	Dispense aliquots into 2 mL microtubes:	1 mL	1 mL	30 μ L ^a	10 μ L ^a
4	Pellet cells, aspirate PBS and resuspend cells with a working siRNA transfection complex volume of:	300 μ L	200 μ L	150 μ L	30 μ L
5	Incubate at room temperature for:	3–5 min	3–5 min	3–5 min	3–5 min
6	Add serum-free media:	300 μ L/well	200 μ L/well	150 μ L/well	30 μ L/well
7	Transfer cells/siRNA transfection complex to each well:	600 μ L/well	400 μ L/well	N/A	N/A
8	Incubate under normal cell culture conditions for:	2–4 hr	2–4 hr	2–4 hr	2–4 hr
9	Add complete growth media:	1 mL/well	670 μ L/well	500 μ L/well	100 μ L/well
10	Incubate under normal cell culture conditions for:	24–72 hr	24–72 hr	24–72 hr	24–72 hr
11	Quantify mRNA and/or protein knockdown as appropriate.				

a. Add cell suspensions directly to the cell-culture plate. Perform all subsequent steps in the cell-culture plate.

Preparing Bulk Working Transfection Complexes

Preparing multiple replicate 3X working siRNA/siRNA transfection reagent complexes in parallel for generating bulk volumes:

Number of 3X Working Transfection Complexes	Combined Bulk Working Transfection Complexes	Wells of a 96-Well Plate	Wells of a 24-Well Plate	Wells of a 12-Well Plate	Wells of a 6-Well Plate
1	1.2 mL	40	8	6	4
2	2.4 mL	80	16	12	8
3	3.6 mL	120	24	18	12
4	4.8 mL	160	32	24	16
5	6.0 mL	200	40	30	20

Contacting Panomics

For technical questions, contact our support group by telephone at (877) 726-6642, or by email at techsupport@panomics.com, or visit our website at www.panomics.com.

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