

Appendix

Table 3. Recommended settings for common cell lines

Cell line	Voltage	Pulse Width	Pulses
CHO-K1	1560	5	10
CHO-S	1650	10	3
U2OS	1320	10	4
Jurkat	1100	20	4
A549	1300	30	1
DU145	1260	20	2
MCF7	1250	20	2
PA317	1320	30	1
HEK293	1200	30	1
C2C12	1650	10	3
HepG2	1050	30	2
NIH-3T3	1400	20	2
SH-SY5Y	1050	30	2

\* The following settings are based on 10 µL tip and the parameters may need to be adjusted depending on the cell condition

Table 4. Recommended number of cells, DNA, and Buffer\* amount per culture vessel for 10 µL tip

Well number	24 well scale				
	1 well	6 wells	12 wells	18 wells	24 wells
Cell number	1.0x10 <sup>5</sup> cells	6.0x10 <sup>5</sup> cells	1.2x10 <sup>6</sup> cells	1.8x10 <sup>6</sup> cells	2.4x10 <sup>6</sup> cells
DNA (1µg/µl)	0.5 µg	3.0 µg	6.0 µg	9.0 µg	12.0 µg
Buffer*	9.5 µL	57.0 µL	114.0 µL	171.0 µL	228.0 µL

Well number	12 well scale		6 well scale	
	1 well	12 wells	1 well	6 wells
Cell number	2.0x10 <sup>5</sup> cells	2.4x10 <sup>6</sup> cells	4.0x10 <sup>5</sup> cells	2.4x10 <sup>6</sup> cells
DNA (1µg/µl)	1.0 µg	12.0 µg	2.0 µg	12.0 µg
Buffer*	9.0 µL	108.0 µL	8.0 µL	48.0 µL

\* Buffer could be either R or T.

Table 5. Recommended number of cells, DNA, and Buffer\* amount per culture vessel for 100 µL tip

Well number	6 well scale		60 mm or 100 mm dish scale	
	1 well	6 wells	60 mm dish	100 mm dish
Cell number	1.0x10 <sup>6</sup> cells	6.0x10 <sup>6</sup> cells	1.0x10 <sup>6</sup> cells	2.0x10 <sup>6</sup> cells
DNA (1µg/µl)	5.0 µg	30.0 µg	5.0 µg	10.0 µg
Buffer*	95.0 µL	570.0 µL	95.0 µL	90.0 µL

\* Buffer could be either R or T.

Table 6. General transfection starting point for siRNA

	96 well	24 well	12 well	6 well
Reagent (µL)	0.125 - 0.2	0.5 - 1	1 - 2	2 - 4
siRNA (pmol)	6.25 - 12.5	25 - 50	50 - 100	100 - 200
Cell density (cells/well)	0.25x10 <sup>5</sup>	1.0x10 <sup>5</sup>	2.0x10 <sup>5</sup>	5.0x10 <sup>5</sup>
Final volume per well (mL)	0.1	0.5	1.0	2.0
Tip	10 µL	10 µL	100 µL	100 µL

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This product is not approved for diagnostic or therapeutic use.

\*Highly recommended to prepare at least 10% extra amount of substances (cells, DNA or siRNA, and buffer) in case of sample loss

Materials Needed

Materials:

1. Healthy cell solution at 1 x 10<sup>7</sup> cells/mL for adherent and 2 x 10<sup>7</sup> cells/mL for suspension
2. 1 - 5 µg/µL high-quality DNA (in DI water or TE buffer) or 100 - 250 µM RNAi duplex (in nuclease-free water)
3. 1.5 mL microcentrifuge tubes
4. Cell culture vessel containing antibiotics-free medium
5. Culture medium containing serum
6. Trypsin/EDTA solution
7. DPBS without calcium and magnesium

Kit:

1. Buffer R or T
2. Buffer E or E2
3. ExTransfection™ tip (10 µL and 100 µL)
4. ExTransfection™ tube

General Guideline

• Note that the transfection efficiency may vary depending on

1. The quality of DNA or siRNA
2. The number of cell passage
3. Confluency of cells

• Discarding period

1. ExTransfection™ tip : after every 2nd use
2. ExTransfection™ tube along with the electrolytic buffer : after every 10th use

NOTE: New tip and buffer should be used every time a new sample is used

• Types of buffers

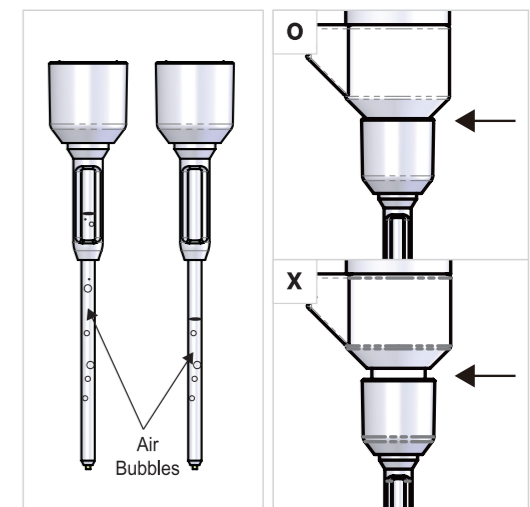
1. Resuspension buffer
    - Buffer R : for mammalian cells
    - Buffer T : for cell types (e.g. blood cells) requiring high voltage protocol of 1900 V or higher
  2. Electrolytic buffer
    - Buffer E : used with 10 µL tip
    - Buffer E2 : used with 100 µL tip
- \*Buffer E and Buffer E2 are not compatible and must not be mixed or diluted together

Pre-Preparation

1. Pre-warm the buffer, culture medium, DPBS, and trypsin/EDTA solution to 37 °C.
  - Buffer E and Buffer E2 are used for 10 µL and 100 µL tip, respectively.
2. Place the ExTransfection™ tube in the ExTransfection™ pipette station after adding proper buffer. \*Avoid spilling any buffer on the station while inserting.

Sample Pre-Preparation

1. Plasmid DNA
    - For optimal result, the size of DNA should not exceed 20 kb.
    - The amount of DNA should not exceed 10% of total volume obtained.
  2. RNAi duplex
    - For optimal result, the concentration of RNAi duplex should be 100 - 250 µM.
    - The amount of siRNA should not exceed 10% of total volume obtained.
- \*Highly recommended to use high-quality materials for optimal transfection and viability.



\* Recommend to prepare at least 10% extra amount of substances (cells, DNA, and buffer) in case of sample loss

Sample Preparation (DNA) \*based on 24 well protocol with 10 µL tip (see Table 1)

1. Prepare cells between 70 % and 90 % confluency. (For adherent cells only)
2. Dissociate the cells. (For adherent cells only)
  - Aspirate the media from the cells.
  - Wash the cells with DPBS.
  - Add enough trypsin/EDTA solution enough to cover the surface to detach cells from the vessel.
3. Put the vessel in the incubator for cell dissociation for no longer than 5 minutes. (For adherent cells only)
4. Meanwhile, prepare a 24-well dish.
  - For 24-well plate, fill each well with 500 µL of antibiotics free culture medium.
  - Then, pre-incubate the plate.
5. Take out the cells and neutralize the trypsin by adding serum-containing medium. (For adherent cells only)
6. Dissociate the cells into single cells, then centrifuge at 100 - 400 g for 5 minutes at room temperature to pellet the cells.
7. Aspirate the media and resuspend the cells with DPBS.
8. Take an aliquot of cell culture and count the cells with a cell counter such as EVE™ / EVE™ Plus or ADAM™ MC2 / MC Plus. Calculate and obtain the required amount of cells.
  - Approximately 2.4 x 10<sup>6</sup> (adherent) or 6.0 x 10<sup>6</sup> (suspension) of live cells should be met.
  - (For other vessel types, refer to appendix.)
9. Centrifuge at 100 - 400 g for 5 minutes at room temperature.
10. Aspirate DPBS then resuspend the cell pellet with Buffer R.
11. Transfer the cells to a new 1.5 mL microcentrifuge tube.
12. Add proper amount of plasmid DNA into resuspended cells.
  - \*Highly recommended to prepare approximately 10% extra amount of substances (cells, DNA or siRNA, and buffer) in case of sample loss

• Table 1. Recommended DNA protocol for various plate formats (per well)

Format	Cell Type	DNA (µg)	ExTransfection™ Tip	Vol. plating medium	Cell no.*	Buffer R or Buffer T <sup>1)</sup>
96-well	Adherent	0.25–0.5	10 µL	100 µL	1–2 × 10 <sup>4</sup>	10 µL/well
	Suspension	0.5–1	10 µL		2–5 × 10 <sup>4</sup>	10 µL/well
48-well	Adherent	0.25–1	10 µL	250 µL	2.5–5 × 10 <sup>4</sup>	10 µL/well
	Suspension	0.5–2	10 µL		5–12.5 × 10 <sup>4</sup>	10 µL/well
24-well	Adherent	0.5–2	10 µL	500 µL	0.5–1 × 10 <sup>5</sup>	10 µL/well
	Suspension	0.5–3	10 µL		1–2.5 × 10 <sup>5</sup>	10 µL/well
12-well	Adherent	0.5–3	10 µL	1 mL	1–2 × 10 <sup>5</sup>	10 µL/well
	Suspension	0.5–3	10 µL		2–5 × 10 <sup>5</sup>	10 µL/well
6-well	Adherent	0.5–3 (10 µL) 5–30 (100 µL)	10 µL/100 µL	2 mL	2–4 × 10 <sup>5</sup>	10 µL or 100 µL/well
	Suspension	0.5–3 (10 µL) 5–30 (100 µL)	10 µL/100 µL		0.4–1 × 10 <sup>6</sup>	10 µL or 100 µL/well
60 mm	Adherent	5–30	100 µL	5 mL	0.5–1 × 10 <sup>6</sup>	100 µL/well
	Suspension	5–30	100 µL		1–2.5 × 10 <sup>6</sup>	100 µL/well
10 cm	Adherent	5–30	100 µL	10 mL	1–2 × 10 <sup>6</sup>	100 µL/well
	Suspension	5–30	100 µL		2–5 × 10 <sup>6</sup>	100 µL/well

\* Recommend to prepare at least 10% extra amount of substances (cells, DNA, and buffer) in case of sample loss

**Sample Preparation (siRNA)** \*based on 24 well protocol with 10 µL tip (see Table 2)

- Cultivate the required number of cells.
- Dissociate the cells.
  - Aspirate the media from the cells.
  - Wash cells with DPBS.
  - Add trypsin/EDTA solution enough to cover the surface to detach cells from the vessel.
- Put the vessel in the incubator for cell dissociation for no longer than 5 minutes.
- Meanwhile, prepare a 24-well plate.
  - For 24-well plate, fill each well with 500 µL of antibiotics free culture medium.
  - Then, pre-incubate the plate.
- Take out the cell and neutralize the trypsin by adding FBS-containing medium.
- Take an aliquot of cell culture and count the cells with a cell counter such as EVE™ / EVE™ Plus or ADAM™ MC2 / MC Plus. Calculate and obtain the required amount of cells.
  - Approximately 2.4 × 10<sup>6</sup> (adherent) or 6.0 × 10<sup>6</sup> (suspension) of live cells should be met.
  - (For other vessel type, refer to appendix.)
- Dissociate the cells into single cells, then centrifuge at 100 – 400 g for 5 minutes at room temperature to pellet the cells.
- Aspirate the media and resuspend the cells with DPBS. Then, obtain the amount calculated from Step 6.
- Centrifuge at 100 - 400 g for 5 minutes at room temperature.
- Aspirate DPBS then resuspend the cell pellet with Buffer R.
- Transfer the cells to a new 1.5 mL microcentrifuge tube.
- Add proper amount of siRNA into resuspended cells.

• Table 2. Recommended siRNA protocol for various plate formats (per well)

Format	Cell Type	siRNA (nM)	ExTransfection™ Tip	Vol. plating medium	Cell no.*	Buffer R or Buffer T <sup>1)</sup>
96-well	Adherent	10–200	10 µL	100 µL	1–2 × 10 <sup>4</sup>	10 µL/well
	Suspension		10 µL		2–5 × 10 <sup>4</sup>	10 µL/well
48-well	Adherent	10–200	10 µL	250 µL	2.5–5 × 10 <sup>4</sup>	10 µL/well
	Suspension		10 µL		5–12.5 × 10 <sup>4</sup>	10 µL/well
24-well	Adherent	10–200	10 µL	500 µL	0.5–1 × 10 <sup>5</sup>	10 µL/well
	Suspension		10 µL		1–2.5 × 10 <sup>5</sup>	10 µL/well
12-well	Adherent	10–200	10 µL	1 mL	1–2 × 10 <sup>5</sup>	10 µL/well
	Suspension		10 µL		2–5 × 10 <sup>5</sup>	10 µL/well
6-well	Adherent	10–200	10 µL/100 µL	2 mL	2–4 × 10 <sup>5</sup>	10 µL or 100 µL/well
	Suspension		10 µL/100 µL		0.4–1 × 10 <sup>6</sup>	10 µL or 100 µL/well
60 mm	Adherent	10–200	100 µL	5 mL	0.5–1 × 10 <sup>6</sup>	100 µL/well
	Suspension		100 µL		1–2.5 × 10 <sup>6</sup>	100 µL/well
10 cm	Adherent	10–200	100 µL	10 mL	1–2 × 10 <sup>6</sup>	100 µL/well
	Suspension		100 µL		2–5 × 10 <sup>6</sup>	100 µL/well

## Transfection

- Add 3 mL of Buffer E into ExTransfection™ tube.

\*Ensure that the electrode at the bottom of the tube is fully submerged in the buffer.

\*Discard the tube after every 10th use.

- Insert the disposable tip securely to the pipette, ensuring the tip is fully pushed in.
  - Push the pipette plunger all the way down to second stop so the clamp opens.
  - Give it a good pressure to securely attach the tip and let go of the plunger gently.
  - The gold electrode of the tip will move up if the tip is correctly clamped.

\*If not fully attached, it may cause an error.

\*Change the tip after every 2nd use or when using different cell type.

- Load the mixture and check to make sure no bubbles are formed inside the tip.
  - To draw up the mixture, push the plunger to the first stop.

\*Fully draw up the mixture once to wet the inner part of the tip and reload.

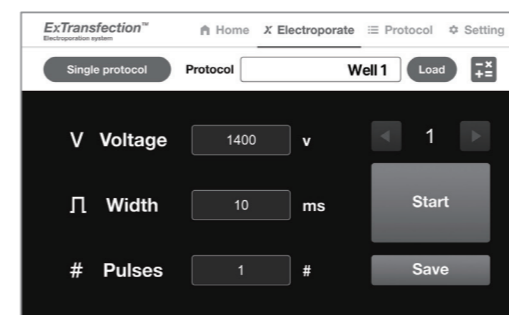
\*Bubbles can cause arcing, leading to inaccurate results and lower transfection rate.

- Insert the pipette into the ExTransfection™ tube.
  - Pipette hook should face towards the station.

\*If not fully inserted, this may cause error.

\*Ensure the tip loaded with the sample does not come into contact with any surface or object.

- Set the appropriate parameters for the cell type. See next page for optimal settings.



① Go to **ELECTROPORATE** tab.

② Enter voltage, width, and pulse values.

- Select START button.

- To use a preprogrammed 24-well optimization protocol, follow the instructions below.
  - Go to ELECTROPORATE tab and select SINGLE PROTOCOL button.
  - For 24-well protocol, select 'Optimization - R buffer'. For 18-well protocol, select 'Optimization - T buffer'.
  - Click APPLY button.
  - Check the parameter values.
  - Then, select START button to proceed.
  - To move to the next well, select  button.

- Once complete, remove the pipette from the station and seed the mixture in the media. Press the plunger only to the first stop to transfer the mixture.

- Discard the tip by pressing the plunger all the way down.

- Repeat Steps 1 to 9 for remaining wells.

- After completing cell seeding, carefully swirl the plate to ensure uniform distribution of the cells.