Torpedo siRNA is a state–of–the–art transfection reagent, specifically designed for the transfer of siRNA and miRNA into a variety of mammalian cell types. It combines high transfection efficiency with low cytotoxicity leading to excellent knockdown even with very small amounts of RNA. In addition to standard formats, such as multiwell plates, Torpedo siRNA is specially engineered for use with ibidi's proprietary plastic material, resulting in fast and efficient gaining of experimental data.

Material

Torpedo siRNA is composed of a proprietary lipid formulation, which results in the efficient and gentle transfer of genetic material into many cell types. Moreover, its special design allows for a transfection protocol with a fixed RNA/reagent ratio, without the need for further optimization. This easy-to-use protocol, where cells are directly added to the Torpedo siRNA mixture, makes it convenient to establish any assay.

Specifications

Packaging and Storage	
Shipping conditions	Room temperature *
Storage conditions	-20°C *
Shelf life	Under proper storage conditions as indicated on vial.
Assays (1.5 ml reagent)	Up to 1500 (96-well), or up to 375 (24-well)

*Has to be completely frozen before first use.

Component	Formulation
Transfection reagent	Proprietary lipids in water
Buffer T	Salt solution, 10 × concentrated

Quality Control

Torpedo siRNA is tested for the absence of microbial contamination with fluid thioglycolate medium, and tested for functionality by using a standard transfection assay.

Important Guidelines

- Determine the siRNA purity by using a OD 260:280 nm ratio. The ratio should be 1:9 or higher. Chemically synthesized siRNAs from commercial suppliers are usually sufficiently pure for transfection.
- Resuspend siRNA with nuclease-free water at a recommended stock concentration of 100 µM. Store aliquots at -20°C.
- RNA is sensitive to RNases. Therefore, appropriate precautions should be taken.
- Use cells that are regularly passaged, proliferating well, and plated at a consistent density.
- Antimicrobial agents (e.g., antibiotics and fungicides), which are commonly included in cell culture media, may adversely affect the transfection efficiency in some cell types.
- Different media and media components may influence the level of transfection efficiency and subsequent growth of the transfected cells, as well as knockdown of the gene of interest.
- Microbial contamination, e.g. Mycoplasma, may decrease transfection efficiency and change experimental outcome.
- When working with a novel cell line, it is important to determine optimal conditions for the highest knockdown efficiency. In principle, this can be analyzed via qPCR (e.g., of human endogenous housekeeping gene HPRT) or Western Blotting (e.g. endogenous Lamin A/C).

Transfection Protocols

For other formats, see "Scaling Up or Down Transfections" (page 6). All amounts and volumes are given on a per well basis.

Both adherent and suspension cells are treated with these protocols.

Note:

The stock solutions of the genetic material, and the transfection reagent, should be at room temperature. Agitate the stock solutions gently before use.

Reverse Protocol in 24-well plate

Cell Preparation

Prepare 500 µl of cell suspension in a complete culture medium with a concentration of 0.8×10^5 cells per ml.

Lipoplex Formation

1. Prepare a 1 × Buffer T by diluting 1 aliquot of 10 × Buffer T with 9 aliquots of sterile water (suitable for cell culture) under sterile conditions.
2. Pipet 60 µl of 1 × Buffer T into a well. Then pipet 4 µl of Torpedo siRNA into the Buffer T, followed by 60 pmol of RNA, also pipetted into the Buffer T. Mix the contents of the well by gentle pipetting.
3. Incubate for 15 mins. at room temperature.
4. Add 500 µl of the prepared cell suspension to the well containing the lipoplex.
5. Incubate the cells, without further mixing, at 37°C in a CO_2 incubator, then assay for gene knockdown after 24 – 72 hours.

Forward Protocol in 24-well plate

Cell Preparation

One day before transfection seed 500 µl per well of the prepared cell suspension (0.8×10^5 cells per ml) in complete culture medium.

Lipoplex Formation

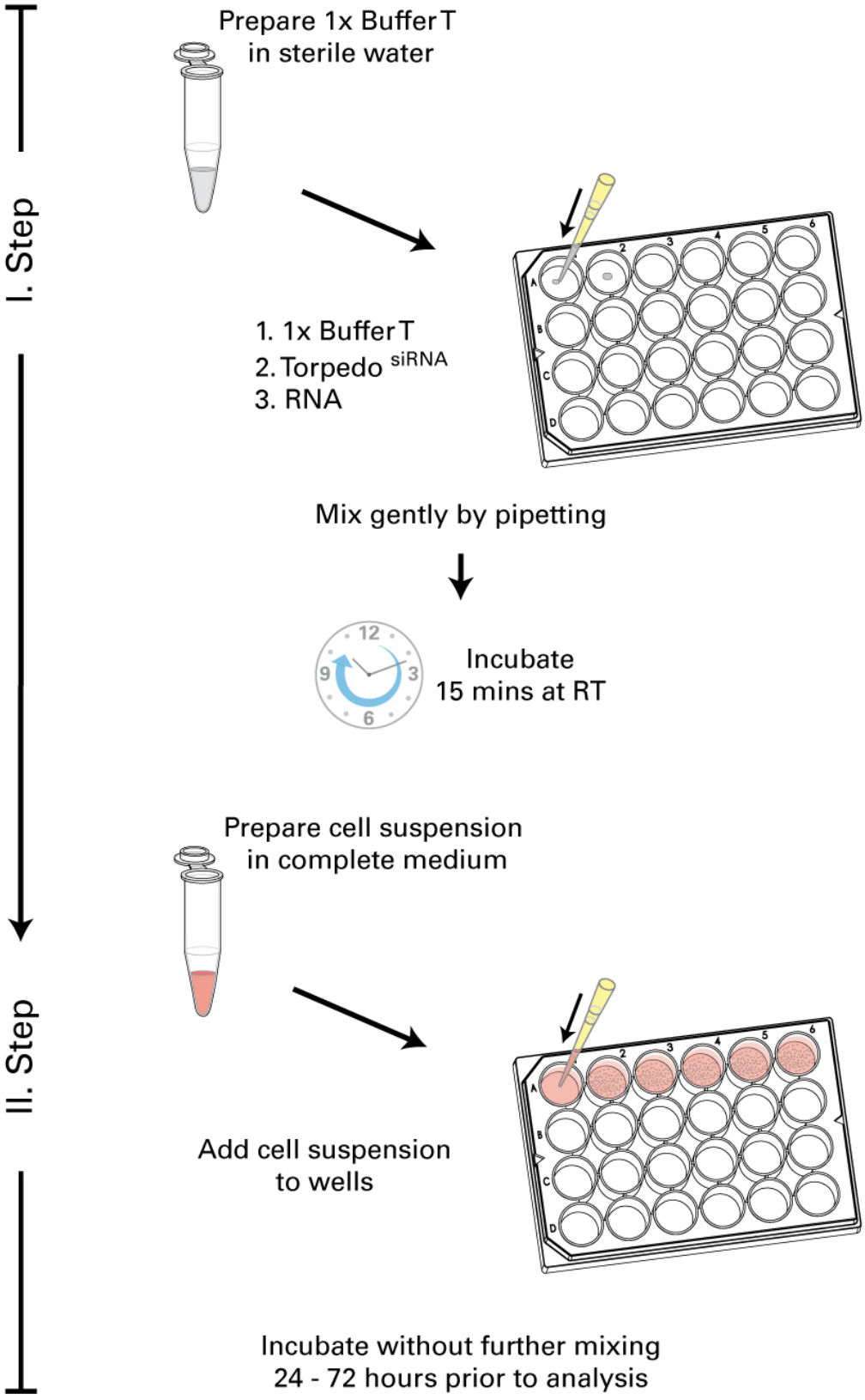
1. Prepare a 1 × Buffer T by diluting 1 aliquot of 10 × Buffer T with 9 aliquots of sterile water (suitable for cell culture) under sterile conditions.
2. Pipet 60 µl of 1 × Buffer T into a 1.5 ml-tube. Then pipet 4 µl of Torpedo siRNA into the Buffer T, followed by 60 pmol of RNA, also pipetted into the Buffer T. Mix the contents of the tube by gentle pipetting.
3. Incubate for 15 mins. at room temperature.
4. Add the lipoplex dropwise to the well containing the cells and gently rock the plate.
5. Incubate the cells, without further mixing, at 37°C in a CO_2 incubator, then assay for gene knockdown after 24 – 72 hours.

Notes:

For extended culturing of transfected cells, it is necessary to change or add fresh culture medium.

Prior to imaging of the cells, it is recommended to wash the wells.

Reverse Protocol for 24-well



Note:

The stock solutions of the genetic material, and the transfection reagent, should be at room temperature. Agitate the stock solutions gently before use.

Reverse Protocol in µ–Slide VI 0.4

Cell Preparation

Prepare 240 µl of cell suspension in a complete culture medium with a concentration of 2 – 3 × 10^5 cells per ml.

Lipoplex Formation

1. Prepare a 1 × Buffer T by diluting 1 aliquot of 10 × Buffer T with 9 aliquots of sterile water (suitable for cell culture) under sterile conditions.
2. Pipet 120 µl of 1 × Buffer T into a 1.5 ml-tube. Then pipet 0.6 µl of Torpedo siRNA into the Buffer T, followed by 10 pmol of RNA, also pipetted into the Buffer T.
   Mix the contents of the tube by gentle pipetting.
3. Incubate for 15 mins. at room temperature.
4. Add 240 µl of the prepared cell suspension to the tube containing the lipoplex.
5. Pipett 30 µl of the mixture into each of the channels of the µ–Slide VI 0.4.
6. Incubate for 6 hours at 37°C in a CO_2 incubator.
7. Wash each of the channels with 120 µl of complete medium.
8. Add 120 µl of complete medium to each channel.
9. Incubate the cells at 37°C in a CO_2 incubator, then assay for gene knockdown after 24 – 72 hours.

Note:

Incubate the slide inside a sterile and humid atmosphere to minimize evaporation until cells have attached and during incubation time. Make sure evaporation is low by using a sterile 10 cm Petri dish with extra wet tissue around the slide.

Forward Protocol in µ–Slide VI 0.4

Cell Preparation

One day before transfection, seed 30 µl per channel of the prepared cell suspension (2 – 3 × 10^5 cells per ml) in complete culture medium. Await cell attachment in order not to flush out the cells. Afterwards fill each reservoir with 60 µl complete medium.
Further information on the µ–Slide VI 0.4 handling at www.ibidi.com.

Lipoplex Formation

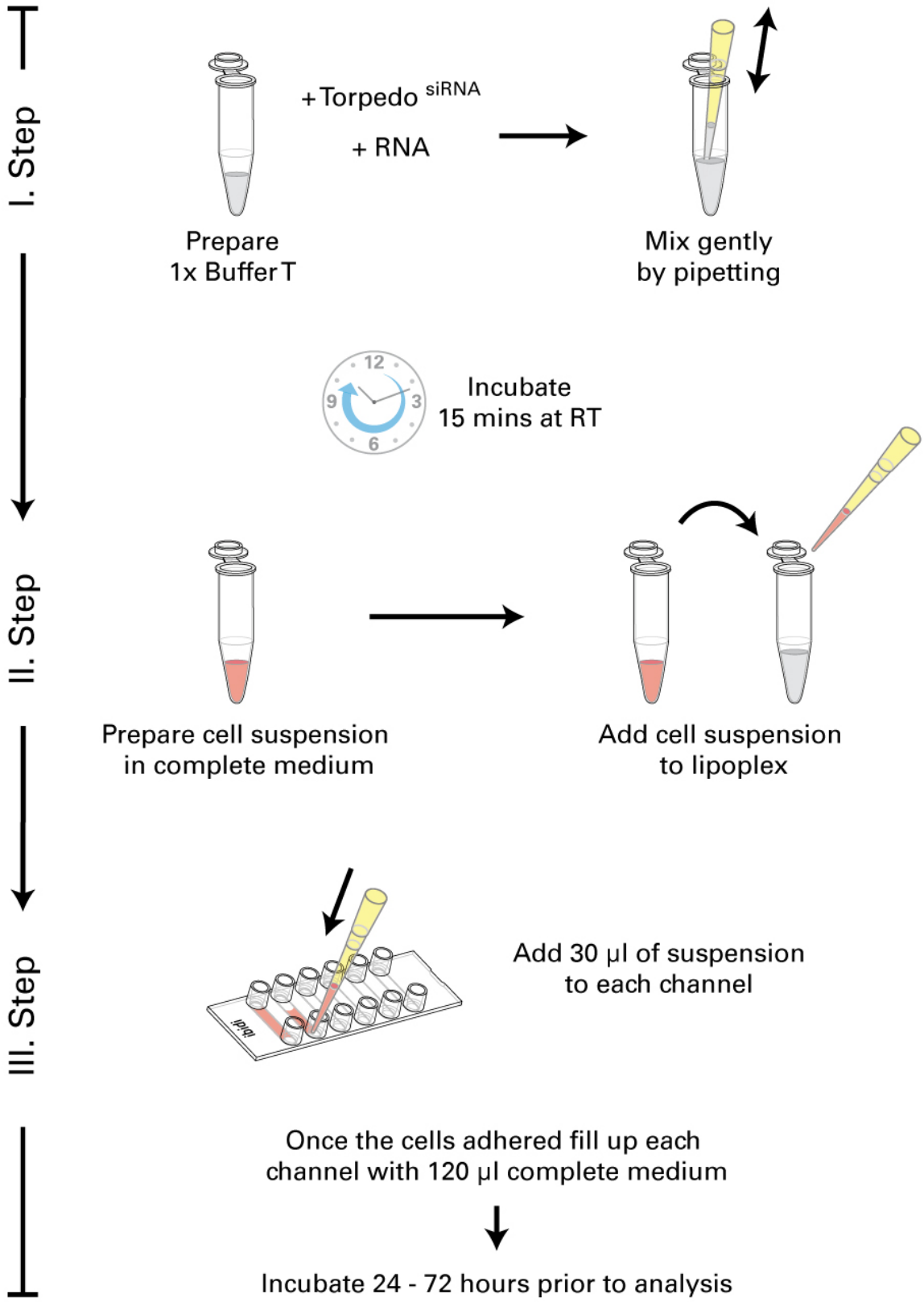
1. Prepare a 1 × Buffer T by diluting 1 aliquot of 10 × Buffer T with 9 aliquots of sterile water (suitable for cell culture) under sterile conditions.
2. Pipet 120 µl of 1 × Buffer T into a 1.5 ml-tube. Then pipet 0.6 µl of Torpedo siRNA into the Buffer T, followed by 10 pmol of RNA, also pipetted into the Buffer T.
   Mix the contents of the tube by gentle pipetting.
3. Incubate for 15 mins. at room temperature.
4. Add 240 µl of complete medium to the tube.
5. Aspirate 120 µl culture medium of each channel and add 60 µl of the lipoplex to one reservoir. Then aspirate 60 µl at the reservoir on the opposite side.
6. Incubate for 6 hours at 37°C in a CO_2 incubator.
7. Replace the lipoplex by washing each channel with 120 µl of complete medium.
8. Add 120 µl of complete medium to each channel.
9. Incubate the cells at 37°C in a CO_2 incubator, then assay for gene knockdown after 24 – 72 hours.

Notes:

For extended culturing of transfected cells, it is necessary to change or add fresh culture medium.

Prior to imaging of the cells, it is recommended to wash the channels.

Reverse Protocol for µ–Slide VI 0.4



Optimizing siRNA/miRNA Transfection

Cell type, siRNA, stability of mRNA, and the targeted protein should always be considered in this process. Depending on the nature of the target gene, transfecting cells at higher densities is also an option when conditions are optimized.

To obtain the highest transfection efficiency and low, non-specific effects, the amount of lipoplex (meaning the amount of Torpedo siRNA and RNA) added to the cells may be changed (e.g. doubled). However, the volumes of the 1 × Buffer T and the cell suspension should not be changed for each culture vessel, and it is especially important to keep the ratio of Torpedo siRNA and RNA constant: 1 µl Torpedo siRNA for every 15 pmol of RNA.

Tips:

- Cells should be 30 – 50% confluent on day of transfection.
- Ensure, that cells are healthy and free of Mycoplasma.
- Antimicrobial agents (e.g., antibiotics and fungicides) which are commonly included in cell culture media, may adversely affect the transfection efficiency in some cell types.
- RNA should be of the highest purity for optimal transfection results.
- Before its use in complex formation with Torpedo siRNA, miRNA/siRNA should not be stored, diluted in medium, for much longer than 5 minutes.
- If toxicity is a problem due to very sensitive cells, remove the transfection mixture after 4 - 6 hours and replace it with medium.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Torpedo siRNA, nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the table.

Culture vessel	Surface area per well*	Buffer T (µl)	RNA (pmol)	Torpedo siRNA (µl)	Cell suspension (ml)
96–well	0.3 cm^2	15	15	1	0.1
48–well	1.0 cm^2	30	30	2	0.25
24–well	1.9 cm^2	60	60	4	0.5
12–well	3.6 cm^2	120	120	8	1
6–well	9.0 cm^2	180	180	12	2
µ–Plate 96 well	0.55 cm^2	20	18	1.2	0.12
µ–Slide 8 well	1.0 cm^2	30	30	2	0.25
µ–Dish 35 mm, high	3.5 cm^2	120	120	8	1

*Surface areas may vary depending on the manufacturer.

When higher cell densities are needed, the amount of lipoplex must be proportionally increased. Remember, it is important to keep the ratio of Torpedo siRNA and RNA constant: 1 µl Torpedo siRNA for every 15 pmol of RNA.

Torpedo family

DNA Transfection

Ordering Number	Labeling	Amount
60610	Torpedo DNA	0.5 ml
60611	Torpedo DNA	1.5 ml
60612	Torpedo DNA	2 × 2.0 ml

siRNA Transfection

Ordering Number	Labeling	Amount
60620	Torpedo siRNA	0.5 ml
60621	Torpedo siRNA	1.5 ml
60622	Torpedo siRNA	2 × 2.0 ml

Additional equipment

µ–Slide 2 well Ph+

Ordering Number	Treatment or Coating
80296	ibiTreat, sterile
80292	Collagen IV, sterile
80293	Fibronectin, sterile*
80294	Poly-L-Lysine, sterile
80295	Poly-D-Lysine, sterile*
80291	uncoated, sterile

* available on request only

µ–Slide 8 well

Ordering Number	Treatment or Coating
80826	ibiTreat, sterile
80822	Collagen IV, sterile
80823	Fibronectin, sterile*
80824	Poly-L-Lysine, sterile
80825	Poly-D-Lysine, sterile*
80821	uncoated, sterile

* available on request only

µ–Slide 4 well Ph+

Ordering Number	Treatment or Coating
80446	ibiTreat, sterile
80442	Collagen IV, sterile
80443	Fibronectin, sterile*
80444	Poly-L-Lysine, sterile
80445	Poly-D-Lysine, sterile*
80441	uncoated, sterile

* available on request only

µ–Slide VI 0.4

Ordering Number	Treatment or Coating
80606	ibiTreat, sterile
80602	Collagen IV, sterile
80603	Fibronectin, sterile*
80604	Poly-L-Lysine, sterile
80605	Poly-D-Lysine, sterile*
80601	uncoated, sterile

* available on request only

µ–Plate 24 well

Ordering Number	Treatment or Coating
82406	ibiTreat, sterile
82401	uncoated, sterile

µ–Plate 96 well

Ordering Number	Treatment or Coating
89626	ibiTreat, sterile
89621	uncoated, sterile

For research use only!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e–mail *info@ibidi.de* or by telephone +49 (0)89/520 46 17 0. All products are developed and produced in Germany.