Torpedo DNA is a state–of–the–art transfection reagent for transfer of nucleic acids (DNA and RNA) into a variety of eukaryotic cell types. It combines high transfection efficiency with low cytotoxicity leading to cells that are ready–to–use for live–cell imaging. In addition to standard formats, such as multiwell plates, Torpedo DNA is particularly engineered for use with ibidi's proprietary plastic materials resulting in fast and efficient gaining of experimental data.

Material

Torpedo DNA is composed of a proprietary cationic lipid formulation resulting in efficient and gentle transfer of genetic material into many cell types. Torpedo DNA mixture can be added directly to cells in culture medium with or without serum. It is not necessary to remove complexes by changing or adding medium after transfection. If very sensitive cells are used, complexes may be removed after 3 – 6 hours.

Specifications

Packaging and Storage	
Formulation	Proprietary cationic lipid formulation.
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 (24–well) or up to 450 (6–well).

*Has to be completely frozen before first use.

Quality Control

Torpedo DNA is tested for absence of microbial contamination with fluid thioglycolate medium, and functionally by a standard transfection assay with a reporter plasmid.

Important Guidelines

- DNA should be of highest purity for optimal transfection results. Use high quality DNA preparation kits to obtain endotoxin–free DNA. Determine DNA purity using 260 nm absorption. The optimal 260 nm/280 nm ratio is between 1.8 and 2.0.
- Use sterile TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) or sterile water for generation of purified plasmid stock (0.1 µg/µl – 2.0 µg/µl).
- Before its use in complex formation with Torpedo DNA, DNA (RNA) should not be stored diluted in medium for longer than 5 min.
- If toxicity is a problem because of very sensitive cells, remove the transfection mixture after 3 – 6 hours and replace it with medium.
- Serum may inhibit complex formation. Use serum– and antibiotic–free medium for lipoplex formation. Once the complex is formed, contact with serum is permitted.
- Opti–MEM® I Reduced Serum Medium (Invitrogen) or serum–free medium is recommended.
- Avoid use of antibiotics during transfection as this may cause cell death.
- Test serum–free media for compatibility with Torpedo DNA since some serum–free formulations (e.g. CD293, SFM II, VP–SFM) may inhibit cationic lipid–mediated transfection.
- We recommend using sterile polypropylene tubes or round–bottom 96–well plates for lipoplex formation
- Quantify cell number to maintain same seeding conditions.
- Do not use siliconized pipette tips or tubes.

Plasmid DNA Transfection in µ–Slide 8 well

For other formats, see "Scaling Up or Down Transfections" (page 3). All amounts and volumes are given on a per well basis.

Cell Preparation

For adherent cells: Seed 2.0 – 10.0 × 10^4 cells per well in 300 µl culture medium (0.7 – 3.3 × 10^5 cells per ml) without antibiotics one day before transfection.

For suspension cells: Seed 0.8 – 4.0 × 10^4 cells per well in 300 µl culture medium (0.3 – 1.3 × 10^5 cells per ml) without antibiotics on day of transfection.

Lipoplex Formation

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

1. Prepare the following solutions while starting with medium: Use serum– and antibiotic–free medium here!

   a) Solution I: 0.04 – 0.5 µg of DNA (RNA[1]) in 30 µl medium.

   b) Solution II: 0.2 – 3.5 µl of Torpedo DNA in 30 µl medium.

   Mix each solution gently.

2. Combine the two solutions by adding Solution I to Solution II. Mix gently and incubate at room temperature for 15 – 20 min.

3. Add the DNA– (RNA[1]–) lipid complexes dropwise to the cells and gently rock the flask. Incubate at 37°C in a CO_2 incubator for approximately 18 – 72 h prior to analysing cell transfection.

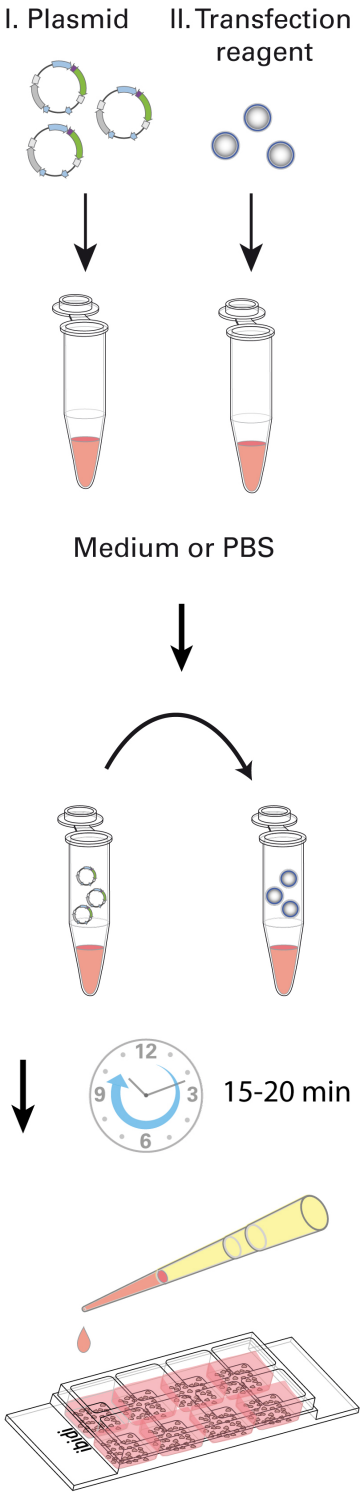
For stable cell lines: Add selective medium (if desired, e.g. G418) into fresh growth medium 24 – 72 hours after transfection.

Note:

Instead of serum– and antibiotic–free medium, 1× PBS can also be used in DNA–lipid complex formation. This could improve reproducibility and may lead to higher transfection rates, particularly with lower volumes of lipids.

[1]RNA means single-stranded RNA and not siRNA.

Protocol for µ–Slide 8 well



Optimizing Plasmid DNA Transfection

To obtain the highest transfection efficiency and low cytotoxicity, optimization of transfection conditions may be necessary.

Tips:

- Cells should be 90 – 100% confluent on day of transfection. Lower cell densities were successfully used but may require different optimization parameters.
- Ensure, that cells are healthy and free of mycoplasma.
- Antimicrobial agents (e.g., antibiotics and fungicides) commonly included in cell–culture media may adversely affect the transfection efficiency in some cell types.
- Verification of the integrity of the vector should be done in advance.
- The DNA (µg) : Torpedo DNA (µl) ratio should be tested from 1:1 to 1:7.
- To avoid adversely affecting transfection efficiency, do not allow undiluted Torpedo DNA to come into contact with plastic surfaces. Do not use siliconized pipette tips or tubes.
- Serum may inhibit complex formation. Once the complex is formed, contact with serum is permitted.
- Make sure that evaporation is minimized when using ibidi slides. Place the µ–Slides each in an extra humidity chamber (petri dish with pieces of water soaked paper towel) inside the incubator. Use sterile water and tissue.
- Follow the instructions of ibidi µ–Slides and µ–Dishes for correct handling and to obtain optimal transfection results.

Scaling Up or Down Transfections

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

Culture vessel	Surface area per well*	Volume of plating medium	Volume of dilution medium**	DNA	Torpedo DNA Ratio 1:3	Torpedo DNA Ratio 1:5
96–well	0.3 cm^2	150 µl	2 x 30 µl	0.1 µg	0.3 µl	0.5 µl
24–well	1.9 cm^2	500 µl	2 x 40 µl	0.5 µg	1.5 µl	2.5 µl
6–well	9.0 cm^2	2.0 ml	2 x 100 µl	2.0 µg	6.0 µl	10.0 µl
µ–Plate 96 well	0.55 cm^2	250 µl	2 x 25 µl	0.2 µg	0.6 µl	1.0 µl
µ–Slide 8 well	1.0 cm^2	300 µl	2 x 30 µl	0.3 µg	0.9 µl	1.5 µl
µ–Dish 35 mm, high	3.5 cm^2	1.8 ml	2 x 100 µl	0.7 µg	2.1 µl	3.5 µl

*Surface areas may vary depending on the manufacturer.
**Volumes of dilution medium in Step 1a and 1b of DNA transfection protocols.

Torpedo DNA family

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

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 4617 0. All products are developed and produced in Germany.