

# LIC™ Transfection Reagent

(Version 2008.6)



Amount: 1 mg lipid (1mg/mL solution in 1.5mL tube)

Store at 2 to 8°C in the dark. Do not freeze.

This product is distributed for *in vitro* laboratory use only.

NOT FOR USE IN HUMANS . NOT FOR DIAGNOSTIC USE.

## General features

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LIC™ Reagent was developed for the transfection of single- or double-stranded RNA into the cytosol of adherent mammalian cells. LIC™ Reagent is a proprietary liposomal formulation of a new cationic lipid analogue and a natural lipid providing the following advantages:

- It has low cytotoxicity.
- The optimal ratio of LIC™ Reagent to RNA has already been determined at 16:1 (w/w).
- It can be added to culture medium containing serum.
- It is not necessary to remove the RNA/ LIC™ complex from the culture medium after transfection.

Cell lines successfully transfected by Nippon Shinyaku: A431, A549, OS-RC-2, A375, AsPC-1, PC-3, MDA-MB-231, MDA-MB-468, HT1080, 22Rv1, LoVo, MCF-7, MCAS, SK-BR-3, Ls174T, 5637, A172, A204, A427, CALU6, DLD-1, HCT15, HT144, KB, RKO, SW480, T47D, UM-UC-3, A673, HCT116, OVCAR-3, COLO-320, MIA-PaCa-2, NIH3T3, B16BL6.

## Storage and stability

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Store LIC™ Reagent at 2 to 8°C in the dark before use with the polypropylene cap tightly closed.

LIC™ Reagent is stable through the expiration date printed on the box when stored at 4°C. After open the cap LIC™ Reagent can be stored for up to 12 months at 2 to 8°C. Contamination by nucleases, microorganisms and chemicals should be avoided. A stability study is ongoing.

## Preparation of LIC™ solutions and RNA/ LIC™ complex for mammalian adherent cells

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1. Storage of LIC™ Reagent
  - LIC™ Reagent must be stored at 2°C to 8°C.
2. Preparation of RNA/ LIC™ complex (RNA/ LIC™ solution)
  - The RNA/ LIC™ solution must be prepared fresh just before use and must be used on the day of preparation. Do NOT store the RNA/ LIC™ solution overnight.
  - The maximum concentration of the RNA/ LIC™ solution must be 50 µg/mL (in terms of RNA) and the minimum volume must be 100 µL.
  - The ratio of RNA to LIC™ must be 1:16 (w/w).
  - Equal volumes of RNA solution and LIC™ working solution must be used, 1:1 (v/v).
  - a. Slowly add the RNA solution to an equal volume of LIC™ working solution. Gently mix the RNA/ LIC™ solution by hand for 5 seconds.
  - b. Further dilute the RNA/ LIC™ solution with RNase-free saline or serum-free culture medium as necessary. Recommendation: Use sterile polystyrene tubes to form the RNA/ LIC™ complex.
3. Culture and treatment of cells
  - a. Seed the cells and incubate them for one day. It is desirable to have 50–60% confluence after one day.
    - Optimization of cell density may be necessary.
  - b. Change the culture medium and add RNA/ LIC™ solution in a volume equal to 1/9 the volume of the culture medium.
  - c. Place the culture plates in a cell-incubator. Further culture medium change is not necessary.
4. Precautions
  - All procedures must be carried out under sterile conditions.
  - The RNA/ LIC™ solution described in this protocol is for *in vitro* experiments only. Do NOT use this RNA/ LIC™ solution for *in vivo* experiments. For *in vivo* experiments, a specific formulation of the RNA/ LIC™ complex is needed.

Note: LIC™ REAGENT IS NOT FOR USE IN HUMANS OR ANIMALS AND NOT FOR DIAGNOSTIC USE.

5. An example of the preparation of RNA/ LIC™ complex

Preparation of 1 μM siRNA/ LIC™ solution using a 21-base-pair siRNA (double-stranded RNA, molecular weight 13,400).

a. Prepare 2 μM siRNA solution (A) and LIC™ working solution (B) as follows.

(A): 2 μM siRNA solution (50 μL)

20 μM siRNA solution	5 μL (1.34 μg RNA)
RNase-free saline or serum-free culture medium	45 μL

(B): LIC™ working solution (50 μL)

LIC™ Reagent (1 mg lipid/mL)	21.4 μL (21.4 μg lipid)
RNase-free saline or serum-free culture medium	28.6 μL

b. Slowly add solution (A) to an equal volume of solution (B). Gently mix the RNA/ LIC™ solution by hand for 5 seconds.

c. Further dilute the RNA/ LIC™ solution with RNase-free saline or serum-free culture medium as necessary. Change the culture medium of the cells. Add the RNA/ LIC™ solution to the cells in a volume equal to 1/9 the volume of the culture medium.

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### Caution

LIC™ Reagent may be used for *in vitro* research only. It may not be used in humans or animals or for diagnostic purposes. Handle the Reagent wearing suitable protective equipments to avoid exposure. Care should be taken to avoid contamination of the product by nucleases or microorganisms by conducting experiments under sterile conditions. For further information, please consult the Material Safety Data Sheet (MSDS), available online at <http://www.genixtalk.com>

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### Terms and conditions

The purchaser may use LIC™ Reagent for in-house research only. The purchaser shall not use the Reagent, or permit it to be used, for any commercial purpose, including but not limited to its resale or its use in the manufacture of any product for sale. The Reagent may be subject to changes in the amount and formulation of the product, the size and nature of the vial, or other production details, without prior notice. The purchaser may not make patent applications relating to the Reagent without the prior written consent of Hayashi Kasei Co., Ltd. If the purchaser does not agree to these terms and conditions, the cost of the Reagent will be refunded in full in exchange for the Reagent.

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### Contact

To address any problems, concerns or question you have, our knowledgeable and responsive technical support team is available daily from 9am to 5:30pm

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