Cat # SL100568-JURKAT Store at 4 °C

GenMute™ siRNA Transfection Reagent for Jurkat Cell

----- A General Protocol for Transfecting siRNA to Jurkat Cell

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

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This product is for laboratory research ONLY and not for diagnostic use

Introduction:

GenMute™ Reagent is a novel biodegradable polymer based siRNA and DNA transfection reagent. With our proprietary pH Dependent Conformational Change (PDCC) technology, the biodegradable polymer was chemically modified by addition of pre-screened hydrophobic groups to side chain, making GenMute™ Reagent the most powerful siRNA delivery tool. GenMute™ siRNA Transfection Reagent for Jurkat is pre-optimized for transfecting siRNA to Jurkat cell with maximum silencing.

Important Guidelines for Transfection:

- Maintain the same seeding conditions between experiments. Use low-passage cells and make sure that cells are healthy and greater than 90% viable before transfection.
- For maximum gene silencing, we recommend using GenMute™ Transfection Buffer to dilute siRNA/DNA and GenMute™ Reagent.
- While the standard protocol for siRNA transfection to Jurkat cell is being given below, optimization is sometimes needed for different siRNAs.

Standard siRNA Transfection of Jurkat Cell

Step I. Preparation of Working Solution of GenMute™ Transfection Buffer:

GenMute™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH_2O into a sterile bottle. The working solution is table at 4 °C~RT for 12 months.

Step II. Transfection of Jurkat Cells:

Use this procedure to transfect siRNA into Jurkat cells in a 24-well format. For other formats, see **Scaling Up or Down Transfections** below. All amounts and volumes are given on a per well basis.

- The day of transfection, count the cells to determine culture density. Plate $1x10^5$ cells per well in 0.5 ml of complete growth medium. Cell density should be ~70% confluent on the day of transfection.

  Note: GenMute™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.
- For each well of cells to be transfected, dilute 20 pmoles siRNA with 50 µl working solution of GenMute™ transfection buffer prepared from **Step I**. Pipette up and down to mix.

  Note: For maximum gene silencing, dilute siRNA reagent with GenMute™ Transfection Buffer (1x).
  We strongly suggest reconstituting siRNA stock solution at 10 µM, so add 2.0 µl siRNA stock solution per well of 24-well plate to make final 40 nM siRNA.
- Add 2.0 µl of GenMute™ Reagent directly to the diluted siRNA solution followed by mix gently and incubate for ~15 minutes at RT.

  Note: Never keep the complex longer than 30 minutes.
- Add the 50 µl transfection mix to the cells drop wise. Gently rock the plate back and forth and return the plate to CO2 incubator.
- Replace transfection medium by cell growth medium ~5 hours after transfection when necessary.
- Gene silencing is usually measured 24~48 hours post transfection.

Storage: GenMute™ siRNA Transfection Reagent is stable for up to 12 months at 4 °C. This item shipped at ambient temperature

Scaling Up or Down Transfections

To transfect Jurkat cells in different tissue culture formats, refer to the table below (Given on a per well basis).

Culture Vessel	Growth Medium (mL)	Cells per Well	Transfection Buffer (µL)	siRNA (pmoles) Final 40 nM	GenMute™ Reagent (µL)
96-well	100	2×10^4	10	4	0.4
24-well	500	1×10^5	50	20	2.0
12-well	1.0	2×10^5	75	40	4.0
6-well	2.0	5×10^5	100	80	8.0
60 mm	4.0	8×10^5	300	160	16
10 cm/T-75 lask	8.0	2×10^6	800	320	32