NutriCulture EcoTRAX Transfection Reagent

100 µl, 3x100 µl

Cat No: ETRA1, ETRA3

Shipping : Ship with blue ice.
Storage : Store at -20°C.

General Information

NutriCulture EcoTRAX Transfection Reagent is formulated as a powerful transfection reagent that ensures effective and reproducible transfection with low cytotoxicity. After *NutriCulture* EcoTRAX Transfection Reagent and plasmid or siRNA or microRNA mixed, the *NutriCulture* EcoTRAX Transfection Reagent protects its cargo from degradation and facilitate efficient delivery into eukaryotic cells. The entire procedure can be completed in 30 minutes.

Important Notes

- To achieve high efficiency and low cytotoxicity, transfection of cells at 50~60% confluency is highly recommended.
- Same seeding conditions between experiments should be maintained.
- Different cell types or number of passages might lead to different transfection efficiency and cytotoxicity. Therefore, at least two different concentrations of transfection reagent as control should be used to optimize experimental conditions when a new cell line or cells at different passages are used.
- Endotoxin-contaminated DNA results in inefficient transfection and can cause high cellular toxicity.

Protocol for Transfection of Plasmids

1. Seed 2000-5000 cells per well in 100 µl medium in a 96-well plate. Incubate cells at 37°C in a humidified CO_2 incubator for 16-24 hours to achieve around 50-60% confluency.

Note: *Culture media with serum usually does not affect transfection efficiency. However, it is not recommended to use antibiotics in growth medium during transfection, since cells are more permeable to antibiotics, which may cause toxicity.*

2. Prepare Mixture 1 with plasmid DNA by diluting it in the appropriate amount of medium (See Table 1). Mix well.

Note: *Plasmid DNA for transfection should be with high purity (A260/A280=1.8-1.9) to ensure efficient transfection mixture preparation.*

Table 1. Transfection amounts for plasmids

	Mixture 1		Mixture 2	
Culture Dish/Plate	Media Volume	Plasmid	Serum-Free Medium	EcoTRAX
96-Well	100 µL	250 ng	10 µL	0.75 µL
24-well	500 µL	500 ng	25 µL	1.5 µL
12-well	700 µL	750 ng	35 µL	2.25 µL
6-well	1 ml	1 µg	50 µL	3 µL
6 cm	3 ml	2.5 µg	150 µL	7.5 µL
10 cm	6 ml	5 µg	300 µL	15 µL

3. Prepare Mixture 2 with *NutriCulture* EcoTRAX by diluting in medium (See Table 1) and incubate 5 minutes at room temperature.

4. Combine Mixture 1 and Mixture 2 in a single microcentrifuge or falcon tube. Then incubate at RT for 25 minutes.

5. Add DNA/EcoTRAX complex into cell culture dish/plate. The mixture can be removed after 6 to 24 hours via refreshing the culture medium.

Note: *For longer incubation periods, please make sure that enough cell culture medium is present in the wells. If not, add appropriate amount of complete culture medium to the wells to make sure the healthy maintenance of the cells.*

6. Carry out further functional tests.

Protocol for Transfection of siRNAs/miRNAs

1. Seed 2000-5000 cells per well in 100 µl medium in a 96-well plate. Incubate cells at 37°C in a humidified CO_2 incubator for 16-24 hours to achieve around 50-60% confluency.

Note: *The medium should be refreshed 30 min before transfection. Usually, culture media with serum does not affect transfection efficiency. However, it is not recommended to use antibiotics in growth medium during transfection, since cells are more permeable to antibiotics, which may cause toxicity.*

2. Prepare Mixture 1 with siRNA/miRNA by diluting it in the appropriate amount of medium (See Table 2). Mix well.

Table 2. Transfection amounts for siRNA/miRNA

	Mixture 1		Mixture 2	
Culture Dish/Plate	Media Volume	siRNA/miRNA (10 µM)	Serum-Free Medium	EcoTRAX
96-Well	25 µL	0.5 µL (5 pmol)	25 µL	1.5 µL
24-well	50 µL	1 µL (10 pmol)	50 µL	3 µL
6-well	150 µL	3 µL (30 pmol)	150 µL	7.5 µL

3. Prepare Mixture 2 with *NutriCulture* EcoTRAX by diluting in medium (See Table 1) and incubate 5 minutes at room temperature.

4. Combine Mixture 1 and Mixture 2 in a single microcentrifuge or falcon tube. Then incubate at RT for 25 minutes.

5. Add DNA/EcoTRAX complex into cell culture plate wells in the following amounts (See Table 3). The mixture could be removed after 6 to 48 hours via refreshing the culture medium.

Table 3. SiRNA/miRNA lipid complex amounts

Culture Dish/Plate	96-Well	24-well	6-well
siRNA/miRNA and lipid complex	10 µL	50 µL	250 µL
Final siRNA used per well	1 pmol	5 pmol	25 pmol
Final EcoTRAX used per well	0.3 µL	1.5 µL	6 µL

6. Carry out further functional tests.