Lipofectamine™ CRISPRMAX™ Transfection Reagent

invitrogen

QUICK REFERENCE Pub. No. MAN0014545 Rev. B.0

Contents and storage

Cat. No.	Amount		Storage
	CRISPRMAX™ Reagent	Cas9 PLUS™ Reagent	
CMAX00001	0.1 mL	1 × 175 µL	Store at 4°C. Do not freeze.
CMAX00003	0.3 mL	1 × 500 µL	
CMAX00008	0.75 mL	1 × 1.25 mL	
CMAX00015	1.5 mL	2 × 1.25 mL	

Product description

Lipofectamine™ CRISPRMAX™ Transfection Reagent is a proprietary formulation for transfecting Cas9 nuclease/gRNA complex into a wide range of eukaryotic cells. The CRISPRMAX™ Transfection Reagent has low cell toxicity, and provides the cleavage efficiency of electroporation with ease of scalability.

The CRISPRMAX™ Transfection Reagent is compatible with TrueGuide™ Synthetic gRNA, TrueCut™ Cas9 Protein v2, and CRISPR libraries from Thermo Fisher Scientific.

Required materials

- gRNA (0.2–3 mg/mL)
- Cas9 nuclease (1mg/mL)
- Opti-MEM™ I Reduced Serum Medium (Cat. No. 31985)
- Microcentrifuge tubes

Online resources

- Visit thermofisher.com/crisprtransfection for additional information and protocols.
- For support, visit thermofisher.com/support.

Important guidelines

- **Cell density at the time of transfection is critical**. Use cells between 30–70% confluent at time of transfection. Test different cell seeding densities to determine the optimal confluence for transfection.
- Cell seeding number is based on growth rate. Seed fewer cells for fast growing cells.
- Mix solutions well by pipetting up and down, or vortexing.
- Cas9 nuclease/gRNA/Cas9 Plus™ Reagent solution (Tube 1) is stable for up to 2 hours at room temperature.
- Dilute CRISPRMAX™ Reagent with Opti-MEM™ I medium, then mix by briefly vortexing. The diluted reagent (Tube 2) does not require incubation.
- Complexes are made in serum-free medium (e.g., Opti-MEM™ I Reduced Serum Medium) and can be added directly to cells in culture medium, with or without antibiotic.
- It is not necessary to remove complexes or change/add medium after transfection.

Genomic cleavage detection assay

After transfecting cells, perform an assay to detect locus specific cleavage of genomic DNA using the GeneArt™ Genomic Cleavage Detection Kit (Cat. No. A24372).

Limited product warranty and licensing information

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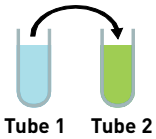
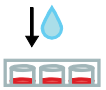
CRISPRMAX™ Reagent Cas9 nuclease transfection protocol for synthetic gRNA

Transfect cells according to the following table. Reaction mix volumes are for one well and account for pipetting variations. Scale the volumes proportionally for additional wells.

IMPORTANT! Prepare solution in Tube 1 before diluting CRISPRMAX™ Reagent in Tube 2. Add reagents in the order indicated in the instructions.

Step			Action			
Day 0	1		Seed cells to be 30–70% confluent at transfection	Component	96-well	24-well	6-well
				Adherent cells	0.8–1.8 × 10^4 cells	4–9 × 10^4 cells	2.5–4.5 × 10^5 cells
Day 1	2	 Tube 1	Mix Cas9 nuclease/gRNA solution with Cas9 Plus™ Reagent **(Tube 1)** Mix well	Component (Tube 1)	96-well	24-well	6-well
				Opti-MEM™ I Medium	5 µL	25 µL	125 µL
				Cas9 nuclease	250 ng	1250 ng	6250 ng
				gRNA (synthetic)	50 ng	240 ng	1200 ng
				Cas9 Plus™ Reagent (add to Tube 1 last)	0.5 µL	2.5 µL	12.5 µL
	3	 Tube 2	Dilute CRISPRMAX™ Reagent in Opti-MEM™ I Medium **(Tube 2)** Mix well	Component (Tube 2)	96-well	24-well	6-well
				Opti-MEM™ I Medium	5 µL	25 µL	125 µL
				CRISPRMAX™ Reagent	0.3 µL	1.5 µL	7.5 µL
				Note: For optimal transfection efficiency, **do not allow** the diluted CRISPRMAX™ Reagent to sit for >3 minutes.			
	4	 Tube 1 Tube 2	Prepare Cas9 nuclease/gRNA/ transfection reagent complex	Immediately add solution from Tube 1 to Tube 2, then mix well.			
	5	 5-10	Incubate	Incubate complex for 5–10 minutes at room temperature. **Do not incubate for >30 minutes.**			
	6		Add complex to cells	Component (per well)	96-well	24-well	6-well
				Cas9 nuclease/gRNA/transfection reagent complex	10 µL	50 µL	250 µL
Day 2–4	7		Visualize/analyze transfected cells	Incubate cells for 2–3 days at 37°C. After incubation, remove culture medium and rinse cells with 50–500 µL PBS, lyse with 20–250 µL lysis buffer, and perform genomic cleavage detection assay.			

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Scaling up or down CRISPRMAX™ transfection reactions for synthetic gRNA

Use the following table to scale the volumes for your transfection experiment according to the type of culture vessel being used.

Culture vessel	Multiplication factor[1]	Starting cell number[2]	Vol. growth medium	Tube 1[3]				Tube 2		Cas9 nuclease/ gRNA/transfection reagent complex
				Vol. Opti-MEM™ I medium	Cas9 nuclease (µg)	gRNA (µg)	Cas9 Plus™ Reagent	Vol. Opti-MEM™ I medium	CRISPRMAX™ Reagent	
96-well	0.17	0.8–1.8 × 10^4	100 µL	5 µL	0.25	0.05	0.5 µL	5 µL	0.3 µL	10 µL
48-well	0.50	2–4.5 × 10^4	250 µL	12.5 µL	0.6	0.12	1.2 µL	12.5 µL	0.8 µL	25 µL
24-well	1.00	4–9 × 10^4	500 µL	25 µL	1.25	0.24	2.5 µL	25 µL	1.5 µL	50 µL
12-well	2.00	8–18 × 10^4	1 mL	50 µL	2.5	0.5	5 µL	50 µL	3 µL	100 µL
6-well	5.00	2.5–4.5 × 10^5	2.5 mL	125 µL	6.25	1.2	12.5 µL	125 µL	7.5 µL	250 µL
60-mm	11.05	4.4–9.9 × 10^5	5 mL	250 µL	13.8	2.8	27.6 µL	250 µL	16.6 µL	500 µL
10-cm	28.95	1.2–2.6 × 10^6	10 mL	500 µL	36.2	7.2	72.4 µL	500 µL	43.4 µL	1 mL
T75	39.47	1.6–3.6 × 10^6	15 mL	750 µL	49.3	9.9	98.7 µL	750 µL	59.2 µL	1.5 mL
T175	92.11	3.7–8.3 × 10^6	35 mL	1.75 mL	115.1	23	230.3 µL	1.75 mL	138.2 µL	3.5 mL

[1] After determining the optimum reagent amount, use the multiplication factor to determine the reagent amount needed for your new plate format.

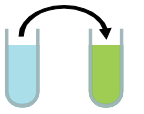
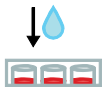
[2] Cell seeding number is based on the cell growth rate. Seed fewer cells for fast growing cells.

[3] The ratio of Cas9 nuclease to gRNA is 5:1 (µg:µg), which is equivalent to a 1:1 molar ratio. The ratio of Cas9 nuclease to Cas9 Plus™ Reagent is 1:2 (µg:µL).

CRISPRMAX™ Reagent Cas9 nuclease transfection protocol for *in vitro* transcribed gRNA

Transfect cells according to the following table. Reaction mix volumes are for one well and account for pipetting variations. Scale the volumes proportionally for additional wells.

IMPORTANT! Prepare solution in Tube 1 before diluting CRISPRMAX™ Reagent in Tube 2. Add reagents in the order indicated in the instructions.

	Step		Action			
Day 0	1 	Seed cells to be 30–70% confluent at transfection	Component	96-well	24-well	6-well
			Adherent cells	0.7–2 × 10^4 cells	0.42–1.2 × 10^5 cells	2.1–6 × 10^5 cells
Day 1	2  Tube 1	Mix Cas9 nuclease/gRNA solution with Cas9 Plus™ Reagent **(Tube 1)** Mix well	Component (Tube 1)	96-well	24-well	6-well
			Opti-MEM™ I Medium	5 µL	25 µL	125 µL
			Cas9 nuclease	105 ng	625 ng	3125 ng
			gRNA (IVT)	21 ng	125 ng	625 ng
			Cas9 Plus™ Reagent (add to Tube 1 last)	0.2 µL	1.3 µL	6.3 µL
	3  Tube 2	Dilute CRISPRMAX™ Reagent in Opti-MEM™ I Medium **(Tube 2)** Mix well	Component (Tube 2)	96-well	24-well	6-well
			Opti-MEM™ I Medium	5 µL	25 µL	125 µL
			CRISPRMAX™ Reagent	0.3 µL	1.5 µL	7.5 µL
			Note: For optimal transfection efficiency, **do not allow** the diluted CRISPRMAX™ Reagent to sit for >3 minutes.			
	4  Tube 1 Tube 2	Prepare Cas9 nuclease/gRNA/ transfection reagent complex	Immediately add solution from Tube 1 to Tube 2, then mix well.			
	5  5-10	Incubate	Incubate complex for 5–10 minutes at room temperature. **Do not incubate for >30 minutes.**			
	6 	Add complex to cells	Component (per well)	96-well	24-well	6-well
			Cas9 nuclease/gRNA/transfection reagent complex	10 µL	50 µL	250 µL
Day 2–4	7 	Visualize/analyze transfected cells	Incubate cells for 2–3 days at 37°C. After incubation, remove culture medium and rinse cells with 50–500 µL PBS, lyse with 20–250 µL lysis buffer, and perform genomic cleavage detection assay.			

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Scaling up or down CRISPRMAX™ transfection reactions for *in vitro* transcribed gRNA

Use the following table to scale the volumes for your transfection experiment according to the type of culture vessel being used.

Culture vessel	Multiplication factor[1]	Starting cell number[2]	Vol. growth medium	Tube 1[3]				Tube 2		Cas9 nuclease/ gRNA/transfection reagent complex
				Vol. Opti-MEM™ I medium	Cas9 nuclease (µg)	gRNA (µg)	Cas9 Plus™ Reagent	Vol. Opti-MEM™ I medium	CRISPRMAX™ Reagent	
96-well	0.17	0.7–2 × 10^4	100 µL	5 µL	0.105	0.021	0.2 µL	5 µL	0.3 µL	10 µL
48-well	0.50	0.21–0.6 × 10^5	250 µL	12.5 µL	0.315	0.063	0.6 µL	12.5 µL	0.8 µL	25 µL
24-well	1.00	0.42–1.2 × 10^5	500 µL	25 µL	0.625	0.125	1.3 µL	25 µL	1.5 µL	50 µL
12-well	2.00	0.84–2.4 × 10^5	1 mL	50 µL	1.25	0.25	2.5 µL	50 µL	3 µL	100 µL
6-well	5.00	2.1–6 × 10^5	2.5 mL	125 µL	3.15	0.63	6.3 µL	125 µL	7.5 µL	250 µL
60-mm	11.05	0.46–1.3 × 10^6	5 mL	250 µL	6.9	1.38	13.8 µL	250 µL	16.6 µL	500 µL
10-cm	28.95	1.2–3.5 × 10^6	10 mL	500 µL	18.1	3.62	36.2 µL	500 µL	43.4 µL	1 mL
T75	39.47	1.66–4.7 × 10^6	15 mL	750 µL	24.65	4.93	49.3 µL	750 µL	59.2 µL	1.5 mL
T175	92.11	0.39–1.1 × 10^7	35 mL	1.75 mL	57.55	11.51	115.1 µL	1.75 mL	138.2 µL	3.5 mL

[1] After determining the optimum reagent amount, use the multiplication factor to determine the reagent amount needed for your new plate format.

[2] Cell seeding number is based on the cell growth rate. Seed fewer cells for fast growing cells.

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