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Package contents

Catalog No.	CRISPRMAX™ Reagent	Cas9 PLUS™ Reagent
CMAX00001	0.1 mL vial	70 µL vial
CMAX00003	0.3 mL vial	200 µL vial
CMAX00008	0.75 mL vial	500 µL vial
CMAX00015	1.5 mL vial	1 mL vial

Storage conditions

Store at 4°C (do not freeze).

Required materials

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

Timing

- Preparation: 10 minutes
- Incubation: 5 + 10 minutes
- Final Incubation: 2–3 days

Selection guide

GeneArt™ CRISPR genome editing

Go online to view related products.

Product description

Lipofectamine™ CRISPRMAX™ Transfection Reagent is a proprietary formulation for transfecting Cas9 nuclease/gRNA complex into a wide range of eukaryotic cells.

Important guidelines

- **Cell density at the time of transfection is critical**. Testing different cell seeding densities may be necessary to determine the optimal confluence for transfection.
- Cell seeding number is based on the cell growth rate. Seed fewer cells for fast growing cells.
- Complexes are made in serum-free medium such as Opti-MEM™ I Reduced Serum Medium and can be added directly to cells in culture medium, with or without antibiotic.
- Cas9 nuclease/gRNA/Cas9 Plus™ Reagent solution (Tube 1) stable for up to 2 hours at room temperature.
- It is not necessary to remove complexes or change/add medium after transfection.

Online resources

Visit our **product page** for additional information and protocols. For support, visit **thermofisher.com/support**.

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Protocol outline

This protocol is compatible with gRNA produced using the GeneArt™ Precision gRNA Synthesis Kit (Cat. no. A29377), and GeneArt™ Platinum Cas9 Nuclease (Cat. nos. B25640 , B25641) or their equivalent.

A. Plate cells so they will be 30–70% confluent at the time of transfection.

B. Prepare Cas9 nuclease/gRNA/transfection reagent complex.

C. Add the complex to cells and incubate for 2–3 days.

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).

Scaling up or down transfections

Click above or go to page 3 for guidelines to scale your transfection experiment according to the type of culture vessel you are using.

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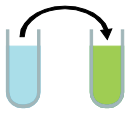
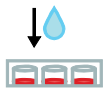
Transfect cells according to the following table. **It is important to add reagents in the order indicated in the instructions**. Prepare Cas9 nuclease/gRNA/Cas9 Plus™ Reagent solution (Tube 1) **before** diluting CRISPRMAX™ Reagent (Tube 2). Mix well by pipetting up and down, or vortexing.

Volumes in each column are for a single well. Scale the volumes proportionally for additional wells. Reaction mix volumes are for one well and account for pipetting variations.

Timeline			Steps	Procedure Details
Day 0	1		**Seed cells to be 30–70% confluent at transfection**	
Day 1	2	 Tube 1	**Prepare Cas9 nuclease/gRNA solution with Cas9 Plus™ Reagent (Tube 1) – Mix well**	
	3	 Tube 2	**Dilute CRISPRMAX™ Reagent in Opti-MEM™ I Medium (Tube 2) – Mix well**	
	4	 5	**Incubate**	
	5	 Tube 1 Tube 2	**Prepare Cas9 nuclease/gRNA/ transfection reagent complex**	
	6	 5-10	**Incubate**	
	7		**Add complex to cells**	
Day 2–4	8		**Visualize/analyze transfected cells**	

Component	96-well	24-well	6-well
Adherent cells	0.7–2 × 10^4	0.42–1.2 × 10^5	2.1–6 × 10^5
Opti-MEM™ I Medium	5 µL	25 µL	125 µL
Cas9 nuclease	85 ng	500 ng	2500 ng
gRNA	21 ng	125 ng	625 ng
Lipofectamine™ Cas9 Plus™ Reagent	0.17 µL	1 µL	5 µL
Opti-MEM™ I Medium	5 µL	25 µL	125 µL
Lipofectamine™ CRISPRMAX™ Reagent	0.3 µL	1.5 µL	7.5 µL

Incubate Cas9 nuclease/gRNA/Cas9 Plus™ solution (Tube 1) and diluted CRISPRMAX™ Reagent in Opti-MEM™ Medium (Tube 2) for 5 minutes at room temperature.

Add solution from Tube 1 to diluted CRISPRMAX™ Reagent in Tube 2, and mix well.

Incubate for 5–10 minutes at room temperature.
Do not incubate for more than 30 minutes.

Component (per well)	96-well	24-well	6-well
Cas9 nuclease/gRNA/ transfection reagent complex	10 µL	50 µL	250 µL

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.

Scaling up or down Lipofectamine™ CRISPRMAX™ Transfections

Use the following table to scale the volumes for your transfection experiment. The most common sizes are listed below.

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	$0.7–2 \times 10^4$	100 µL	5 µL	0.085	0.021	0.17 µL	5 µL	0.26 µL	10 µL
48-well	0.50	$0.21–0.6 \times 10^5$	250 µL	12.5 µL	0.25	0.063	0.5 µL	12.5 µL	0.75 µL	25 µL
24-well	1.00	$0.42–1.2 \times 10^5$	500 µL	25 µL	0.50	0.125	1 µL	25 µL	1.5 µL	50 µL
12-well	2.00	$0.84–2.4 \times 10^5$	1 mL	50 µL	1.00	0.25	2 µL	50 µL	3 µL	100 µL
6-well	5.00	$2.1–6 \times 10^5$	2 mL	125 µL	2.50	0.63	5 µL	125 µL	7.5 µL	250 µL
60-mm	11.05	$0.46–1.3 \times 10^6$	5 mL	250 µL	5.53	1.38	11 µL	250 µL	16.58 µL	500 µL
10-cm	28.95	$1.2–3.5 \times 10^6$	10 mL	500 µL	14.48	3.62	29 µL	500 µL	43.43 µL	1 mL
T75	39.47	$1.66–4.7 \times 10^6$	15 mL	750 µL	19.74	4.93	39.5 µL	750 µL	59.21 µL	1.5 mL
T175	92.11	$0.39–1.1 \times 10^7$	35 mL	1.75 mL	46.06	11.51	92 µL	1.75 mL	138.17 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] In Tube 1, the ratio of Cas9 nuclease to gRNA is 4:1 (µg:µg), while the ratio of Cas9 nuclease to Cas9 Plus™ Reagent is 1:2 (µg:µL).