

# ***TransIT-X2<sup>®</sup> Dynamic Delivery System for CRISPR/Cas9 Ribonucleoprotein (RNP) + DNA Oligo (ssODN) Delivery***

Instructions for use with MIR 6000, 6003, 6004, 6005, 6006, 6010



## **SPECIFICATIONS**

<b>Storage</b>	Store <i>TransIT-X2<sup>®</sup></i> Dynamic Delivery System tightly capped at $-20^{\circ}\text{C}$ . <b>Before each use</b> , warm to room temperature and vortex gently.
<b>Product Guarantee</b>	1 year from the date of purchase, when properly stored and handled.

## **► CRISPR RIBONUCLEOPROTEIN (RNP) + ssODN TRANSFECTION PROTOCOL**

**Fill in volumes below based on culture vessel used for transfection (Table 1).**

### **A. Plate cells**

1. Approximately 18-24 hours before transfection, plate cells in \_\_\_ml complete growth medium. Most adherent cell types should be  $\sim 80\%$  confluent at the time of transfection.

**For adherent cells:** Plate cells at a density of  $0.8\text{--}3.0 \times 10^5$  cells/ml.

**For suspension cells:** Plate cells at a density of  $2.5\text{--}5.0 \times 10^5$  cells/ml.

2. Culture overnight.

### **B. Prepare *TransIT-X2<sup>®</sup>*:RNP complexes (Immediately before transfection)**

1. Warm *TransIT-X2<sup>®</sup>* to room temperature and vortex gently before using.
2. Place \_\_\_ $\mu\text{l}$  of OptiMEM<sup>®</sup> I Reduced-Serum Medium in a sterile tube.
3. Add \_\_\_ $\mu\text{l}$  of a 50  $\mu\text{M}$  guide RNA stock solution (12 nM final concentration per well). Mix gently by pipetting. NOTE: If using 2-part crRNA + tracrRNA, combine at a 1:1 molar ratio and incubate for 10 minutes at room temperature to anneal. Then add to tube containing OptiMEM<sup>®</sup>.
4. Add \_\_\_ $\mu\text{l}$  of a 30  $\mu\text{M}$  Cas9 protein stock solution (6 nM final concentration per well). Mix gently by pipetting.
5. Incubate at room temperature for 10 minutes.
6. Add \_\_\_ $\mu\text{l}$  ssODN (12 nM final concentration per well) to tube containing RNP mixture.
6. Immediately add \_\_\_ $\mu\text{l}$  *TransIT-X2<sup>®</sup>* to the RNP:ssODN mixture. Mix gently by pipetting.
7. Incubate at room temperature for 15 minutes.

### **C. Distribute complexes to cells**

1. Add the *TransIT-X2<sup>®</sup>*:RNP:ssODN complexes (prepared in Step B) drop-wise to different areas of the well. Gently rock plate for even distribution of complexes.
2. Incubate 24-72 hours.
3. Harvest cells and assay as required.

**Table 1.** Recommended starting conditions

Culture vessel	24-well plate	12-well plate	6-well plate
Surface area	1.9 cm <sup>2</sup>	3.8 cm <sup>2</sup>	9.6 cm <sup>2</sup>
Complete growth medium	0.5 ml	1 ml	2.5 ml
Serum-free medium	50 $\mu\text{l}$	100 $\mu\text{l}$	250 $\mu\text{l}$
guide RNA (50 $\mu\text{M}$ stock, 12 nM final in well)	0.12 $\mu\text{l}$	0.24 $\mu\text{l}$	0.6 $\mu\text{l}$
Cas9 Protein (30 $\mu\text{M}$ stock, 6 nM final in well)	0.1 $\mu\text{l}$	0.2 $\mu\text{l}$	0.5 $\mu\text{l}$
ssODN (50 $\mu\text{M}$ stock, 24 nM final in well)	0.12 $\mu\text{l}$	0.24 $\mu\text{l}$	0.6 $\mu\text{l}$
<i>TransIT-X2<sup>®</sup></i> Reagent	1 $\mu\text{l}$	2 $\mu\text{l}$	5 $\mu\text{l}$

### **► Transfection Optimization:**

The 2:1:2 ratio of guide RNA to Cas9 protein to ssODN (12 nM gRNA:6 nM Cas9:12 nM ssODN; final concentration per well) used in this protocol is a starting point for RNP + DNA oligo transfection. Further ratio optimization may be required for some cell types.

For more on transfection optimization, see the *TransIT-X2<sup>®</sup> full protocol (PDF)* or *Tips from the Bench*.

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