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CRISPR/CAS9 – Genome Editing using viral Particles

ViroMag R/L for lentiviral vectors encoding CRISPR/Cas9 plasmids

The following protocol is given for a single well from a 24-well tissue culture plate containing ~1x10^5 cells/well in 400 µL complete culture serum.

If a different culture plate format is used, adjust cell number and reagent amounts according to the table below:

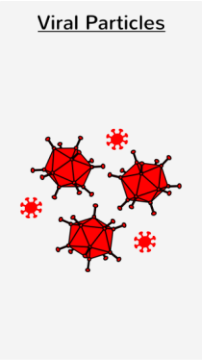
Tissue Culture Dish	Cell Number per well	Total transfection volume per well
96-well	0.5 – 2.0 x 10^4	0.2 mL
24-well	0.5 – 1.0 x 10^5	0.5 mL
12-well	1.0 – 2.0 x 10^5	1.0 mL
6-well	2.0 – 4.0 x 10^5	2.0 mL
60 mm dish	0.5 – 1.0 x 10^6	4.0 mL
90-100 mm dish	1.0 – 2.0 x 10^6	8.0 mL
T75 flask	2.0 – 5.0 x 10^6	12.0 mL

Table 1: recommended cell number and transfection volume.

Key parameter before beginning the procedure:

- The vector (DNA, gRNA, mRNA, protein, viral particles) and transfection reagents solutions should have an ambient temperature and be gently vortexed prior to use.
- All the complexes must be prepared in medium without serum and supplement.
- It is not recommended to use RPMI during complex preparation, prefer DMEM or PBS.
- For sensitive cells, medium can be replaced with fresh complete culture medium 4 to 6h after transfection.

NOTES: ViroMag R/L should be stored at +4°C. Use **6 µL** of ViroMag R/L per well. Viral particles will be diluted in **100 µL** of medium without any supplement (DMEM) and added onto ViroMag R/L magnetic nanoparticles.



Viral vectors encoding CRISPR reagents

1. Lentiviral solutions

The MOI may vary depending on viral construction and cell type: MOI of 3 for HEK-293 cells, and MOI of 60 for iPS. We recommend using a MOI of 10 to begin.

Prepare a viral suspension for a final **MOI=10** in **100 µL** of DMEM without any supplement.

2. ViroMag R/L solution: Add **6 µL** of ViroMag R/L in a new tube.

3. Complexes preparation

A. Add the viral solution onto ViroMag R/L nanobeads, mix gently by carefully pipetting up and down.
B. Incubate the mixture for **20 min** at room temperature.

4. Infection

A. Add the magnetic complexes dropwise onto the cells and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.
B. Place the cells on the magnetic device and incubate at **37°C** for **30 min**.
C. After **30 min** of incubation, remove the magnetic plate.
D. Incubate the cells under your standard culture conditions for **24** to **72 h**.

Optional: perform a medium change. Keep the magnetic plate beneath the cell culture dish, withdraw the transfection medium and add fresh growth medium (optionally without antibiotics). Then, remove the magnetic plate.