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Genome Edited (KO) Cell Line Generation –

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We use this protocol and it's working

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Abstract

This protocol details steps taken to create KO cell lines in RAW 264.7 cells.

Materials

DMEM (Thermo Fisher Scientific, 11965-092)
FBS (Thermo Fisher Scientific, 16140-071)
Penicillin/Streptomycin (Thermo Fisher Scientific, 15140122)
CellStripper (Corning, 356230)
Synthego CRISPR Gene Knockout V2 Mouse Kits
Lipofectamine CRISPRiMAX (ThermoFisher Scientific, CMAX00003)
Cas9 (Synthego CRISPR Gene Knockout V2)
Opti-Mem (Thermo Fisher Scientific, 31985062)

Day 0

1 Plate 2.5 × 10^5 RAW 264.7 cells per well in a 6-well dish.

Day 1

2 Transfect cells using Lipofectamine CRISPRiMAX, gene specific sgRNAs and recombinant Cas9 (Synthego CRISPR Gene Knockout V2).

2.1 **CRISPRiMAX protocol**
In Tube 1 combine 62.5 µL Opti-MEM, 3.25 µL sgRNA (from 3 uM stock), 2.5 µL Cas9 (from 3 uM stock), and 2.5 µL Cas9+ then incubate for 00:05:00. In Tube 2 combine 62.5 µL Opti-MEM and 3.75 µL CRISPRMAX then incubate for 00:05:00. Combine tube 1 and tube 2. Incubate for 00:20:00. Add drop-wise to 1 well containing 1.8 mL of media.

Day 3

3 Change the media.

Daty 4

4 Plate single cells into 96-well dishes to obtain clonal cell lines. After subculturing, confirm cell lines via immunoblotting