

## **si-RNA transfection of mouse bone marrow progenitors by electroporation**

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This protocol was devised for electroporation of mouse bone marrow cells that have been enriched for progenitors by magnetic enrichment for the Sca-1 antigen (using the Miltenyi system). We initially determined optimal electroporation conditions for siRNA by transfecting fluorescently-labeled siRNA oligonucleotides obtained from Orbigen ([www.orbigen.com](http://www.orbigen.com)).

1. Resuspend Sca-1 positive bone marrow cells at  $1 \times 10^6$  cell / ml in DMEM serum-free medium (GIBCO BRL).
2. Place 500  $\mu$ l of the cell suspension in electrode gap cuvettes with 4 mm gap (Life Technologies, Carlsbad, CA). Keep on ice.
3. Add siRNA to the cell suspension to 2  $\mu$ molar final concentration. Gently mix cell-nucleic acid suspension by flicking the cuvette and keep it on ice for 5 minutes prior to electroporation.
4. For electroporation, we use the Life Technologies Cell Porator (Life Technologies, Carlsbad, CA). For temperature control, fill the internal compartment with water at room temperature.
5. Place the electroporation cuvettes in the electroporator and then pulse once at 320 mV, 1600  $\mu$ F.
6. As quickly as possible, add 500  $\mu$ l of long-term bone marrow medium to the chambers\*. Gently transfer the cell suspension in centrifuge tubes and spin down (2000 rpm, 4°C, 8 minutes in a clinical centrifuge).
7. Remove the supernatant and gently resuspend the cell pellet in long-term bone marrow medium.
8. Incubate the cells in a humidified incubator at 37°C, 5% CO<sub>2</sub>.

9. We usually observe RNA interference 3 days after transfection.

To check the viability of the cells after electroporation, take an aliquot and count the cells with trypan blue. Also, an aliquot can be stained with 2 µg/ml of propidium iodide (PI) for dead cell discrimination using FACS analysis. Cell viability immediately after electroporation is typically around 50%, and transfection efficiency is 80%. Efficiency of RNA inhibition can be measured by quantitative real-time PCR or examination for protein product.

**\*Long-term bone marrow medium:**

<b>Component</b>	<b>Final concentration</b>
DMEM medium (high glucose)	base medium
Penicillin/Streptomycin	1%
Horse serum	15%
Fetal calf serum	5%
Hydrocortisone	$10^{-6}$ M
2-Mercaptoethanol	$10^{-4}$ M
Transferrin	400 µg/ml

This medium can be stocked at 4°C for a couple of weeks.

Before using it, add WEHI supernatant (17%, final concentration) and filter.