

Electroporation Buffer for siRNA:

HEPES 21 mM	2.5g
NaCl 137 mM	4.0g
KCl 5 mM	0.186g
Na ₂ HPO ₄ -7H ₂ O 0.7 mM	0.094 g
Dextrose 6mM	0.54 g
H ₂ O	500 ml

(Note, for DNA Pagano's lab has had good results using media (DMEM) without FCS or P/S)

pH 7.15

Filter

Make fresh every 2 weeks

For 2091 fibroblasts

200 V, 500uF

For REF52

300 V, 500 uF

For U2OS

400 V, 960 uF

For HeLa

260V, 850uF

Trypsinize cells (70% confluent 100 mm dish)

Wash in PBS

Resuspend in electroporation buffer (500 ul/100 mm dish)

Place in Cuvette

Add RNAi (100 nM) or DNA (1-5 ug + 20-25 ug carrier DNA without eukaryotic promoter)

Electroporate at above settings

Quickly add to media

Wash cuvette and add contents to media

Evaluate 24-72 hrs later