Lonza Nucleofection Experiment Protocol: For 20 ul rxn chambers used with Lonza 4D nucleofection

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# Prep work:

**Determine rxn conditions for each rxn:**

1. Number of cells/well
2. Amt and volume of substrate
3. Rxn code for nucleofection

**Prepare rxn media and plate for cells post-nucleofection:**

Pre-warm culture media

Pre-warm 1o12w with culture media for each rxn to be performed

**Prepare Substrates and NF reagent:**

1. Dilute substrate into appropriate concentration for each rxn. Ensure volume is above minimum threshold for pipetting, and below 10% of total rxn volume. (**0.5 - 2 ul)**
2. Mix nucleofector **supplement** with nucleofector **solution** using below ratio per 20ul rxn
   1. 16.4 ul solution
   2. 3.6 ul supplement

**Lift, count, and pellet cells:**

1. Trypsinize cells as standard.
2. Dilute, take aliquot for counting.
3. Count total number of cells. Ensure that there are enough cells for 200,000/rxn, or whichever cell number was previously determined.
4. Take the required number of cells.
   1. **spin at 90 x g for 10 min.**
   2. While cells are spinning, ensure that all reagents are prepared for nucleofection.
5. Aspirate media and proceed with nucleofection procedure.

**Nucleofection:**

1. Resuspend cells carefully in prepared nucleofection reagent mix.
2. Aliquot into separate tubes based on the number of rxns.
3. Add substrates to each tube for each rxn.
4. Transfer to nucleofection vessels.
   1. Note: avoid bubbles as much as possible.
5. Tap and cover nucleofection vessels.
6. Proceed with nucleofection. Zap cells.
7. Add media from pre-prepared wells, dilute each rxn and transfer to plate wells.
8. Incubate.