HALO®-96 SPC-QC

A Step by Step Guide to using the HALO®-96 SPC-QC Kit:

For Quality Control of Cord Blood, Peripheral Blood and Bone Marrow for Stem Cell Transplantation and Cord Blood Bank Processing Laboratories.

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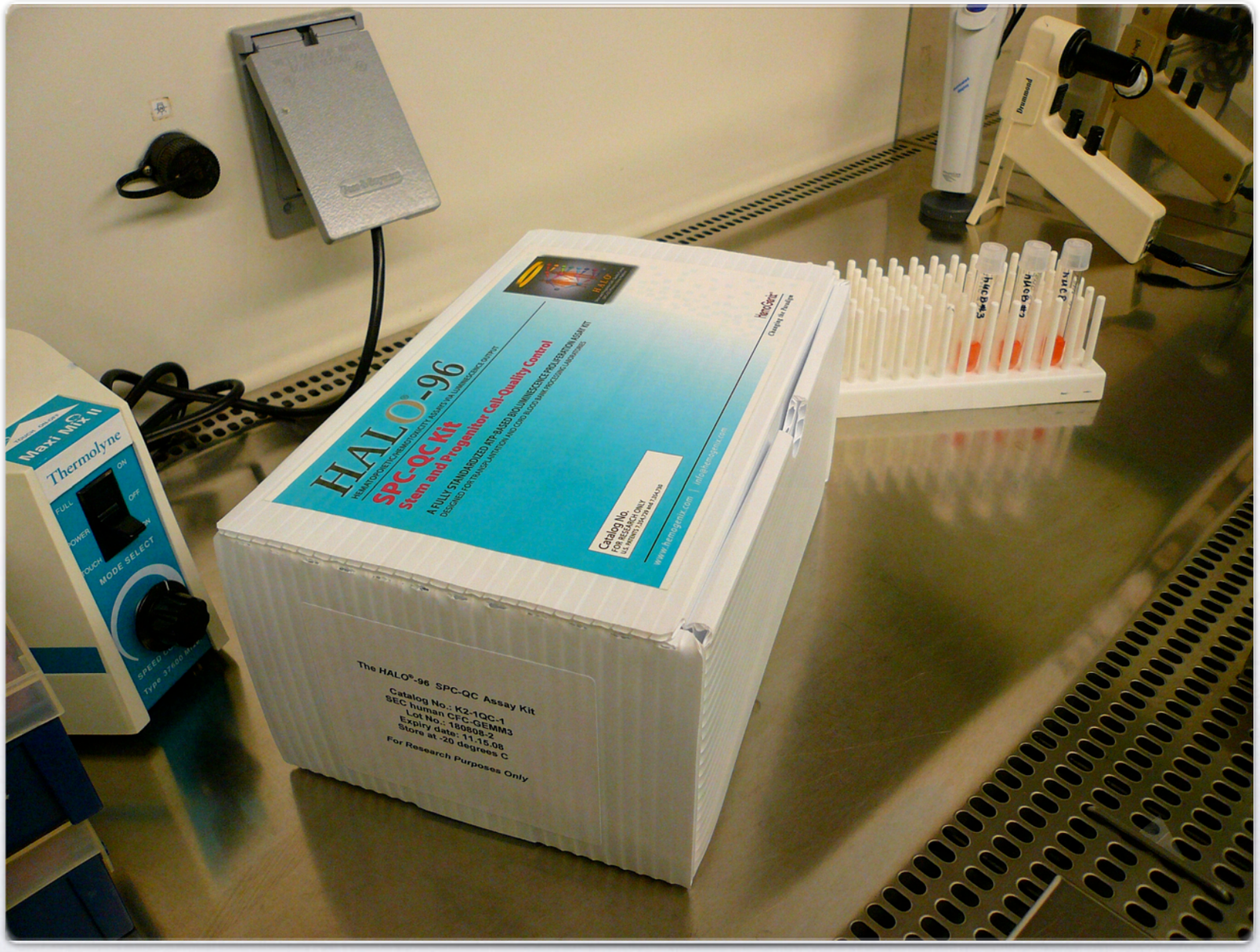
HALO®-96 SPC-QC

Stem and Progenitor Cell - Quality Control

- HALO®-96 SPC-QC is available using methyl cellulose or a liquid Suspension Expansion Culture (SEC)

- We shall be using the SEC kit.



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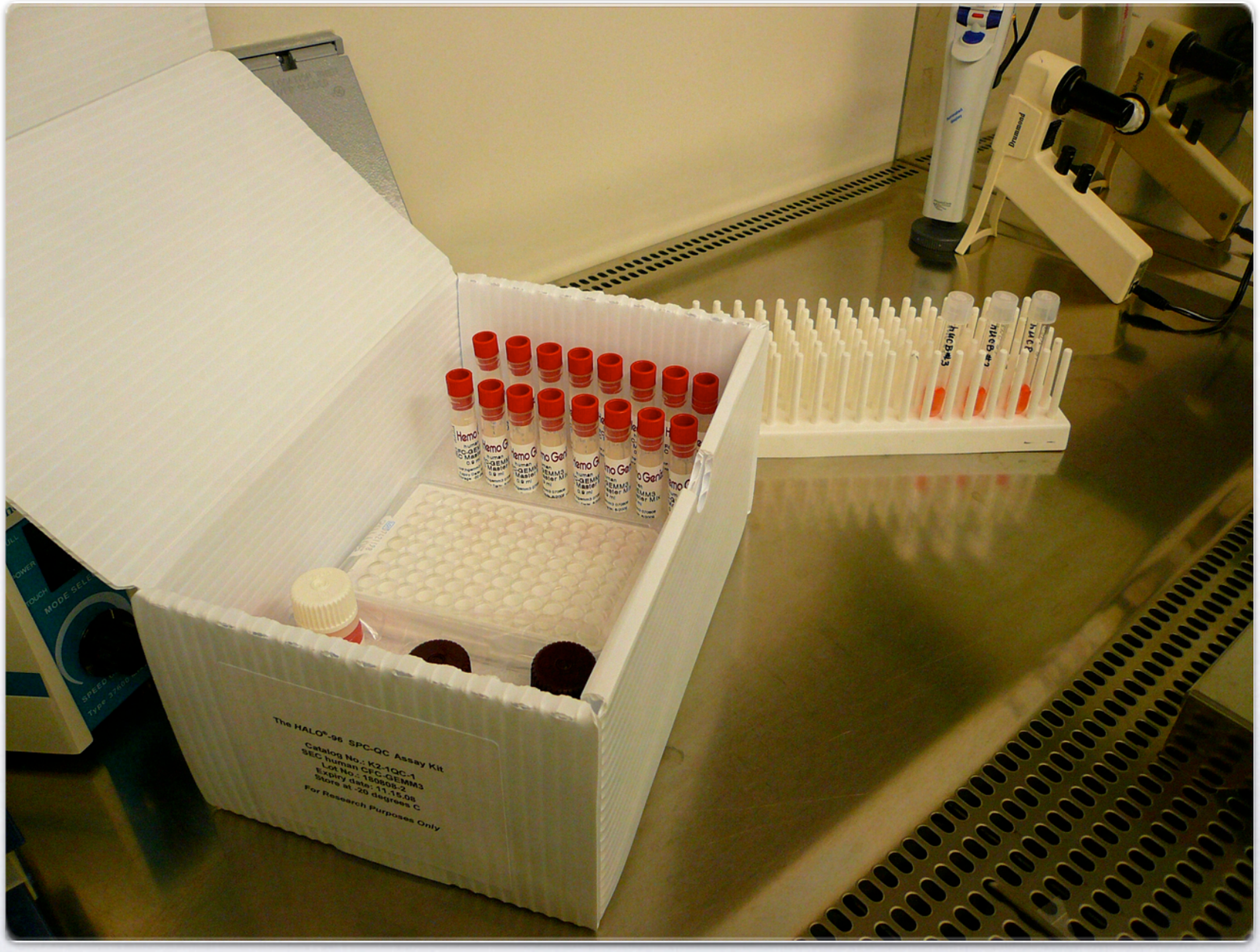
Stem and Progenitor Cell - Quality Control

- The single stem cell (CFC-GEMM) kit can be used for 16 cord blood, peripheral blood or bone marrow samples.



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Stem and Progenitor Cell - Quality Control

- Everything you need for culture and ATP measurement is in the kit. Just add cells.

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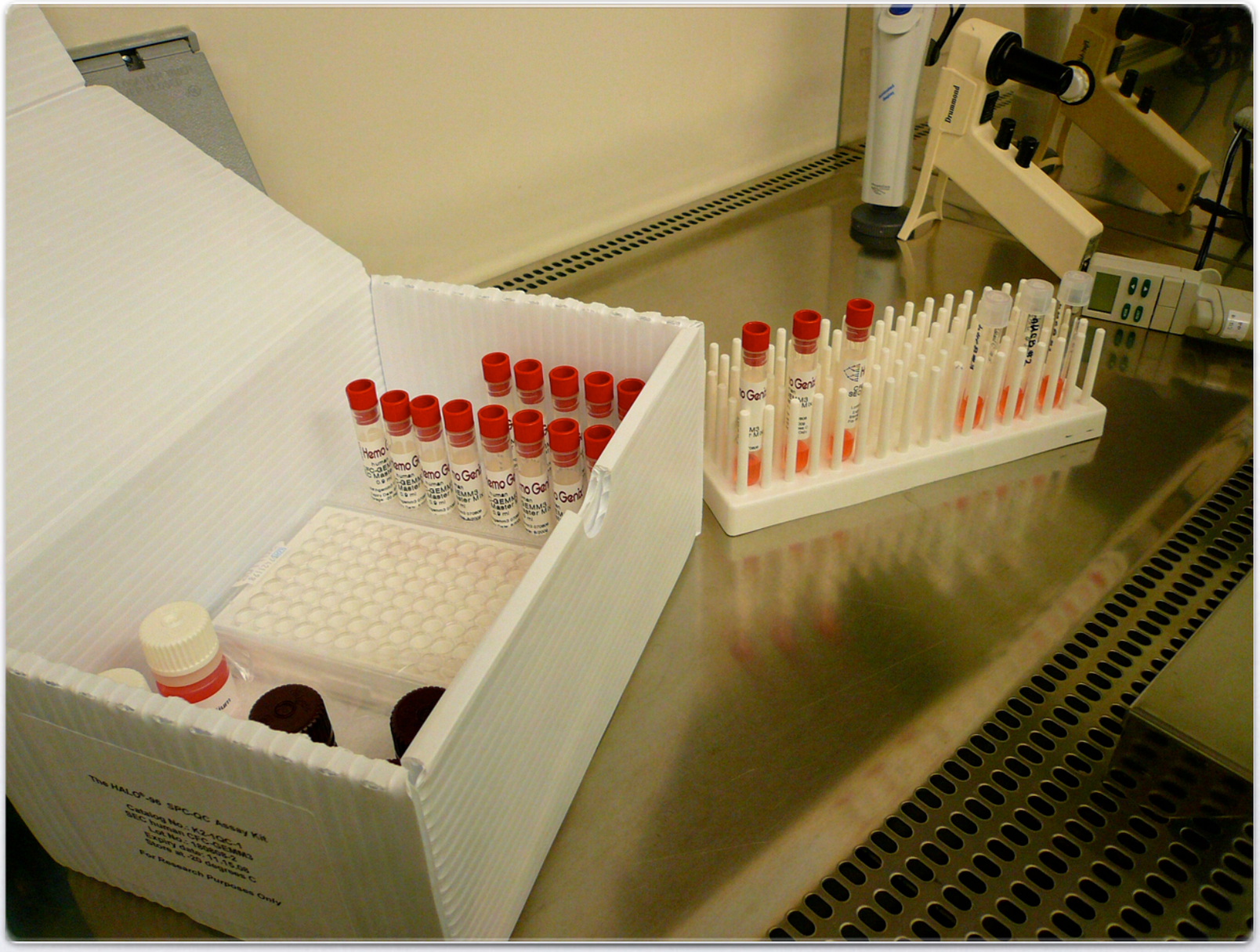
Stem and Progenitor Cell - Quality Control

- We shall be using only 3 of the 16 Master Mix tubes for 3 cord blood samples.

- Remove the tubes and let the contents thaw.



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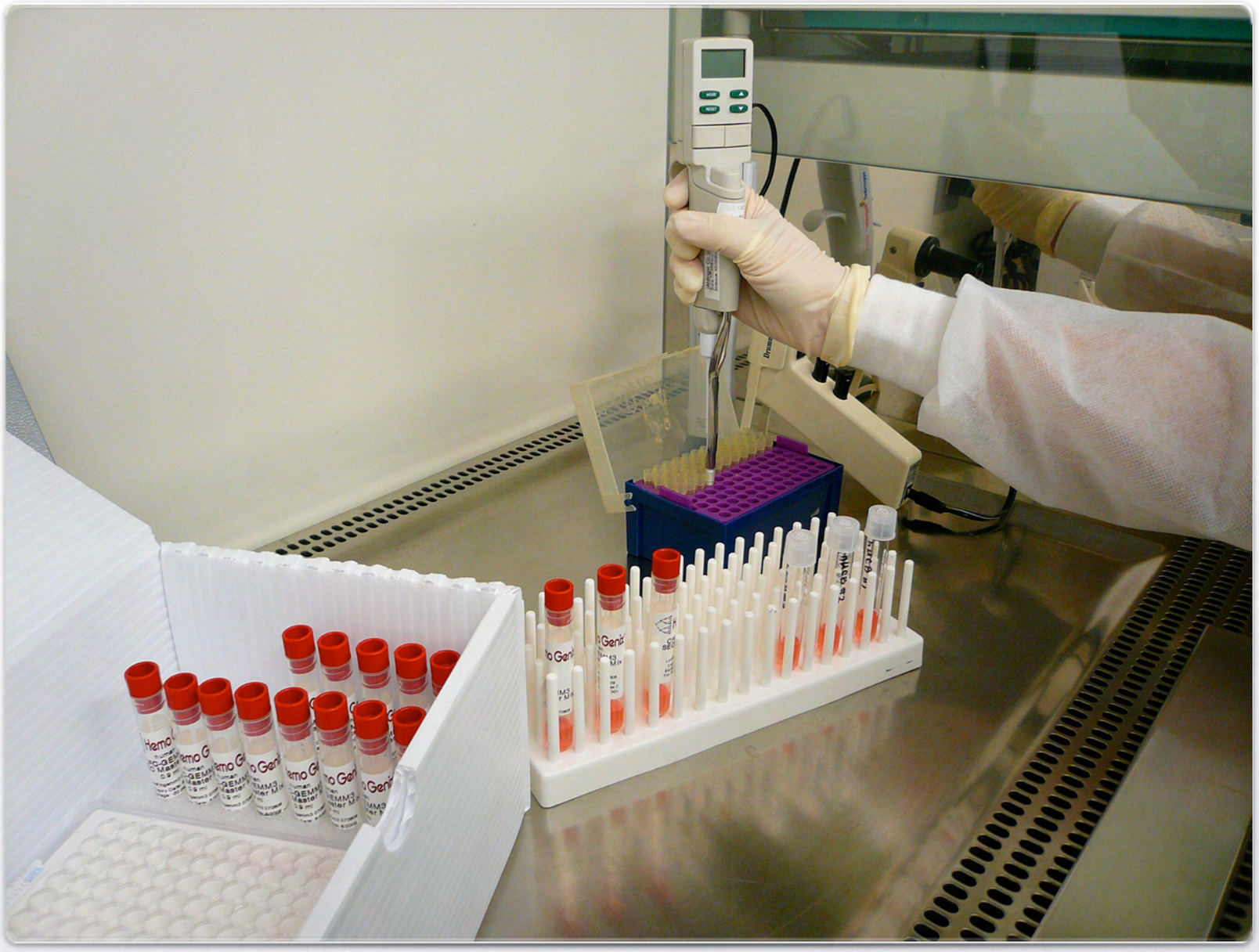
Stem and Progenitor Cell - Quality Control

- Prepare the cell suspension and adjust the concentration of each sample to 500,000 cells/ml.

- This is 100 times the final cell concentration/ well.



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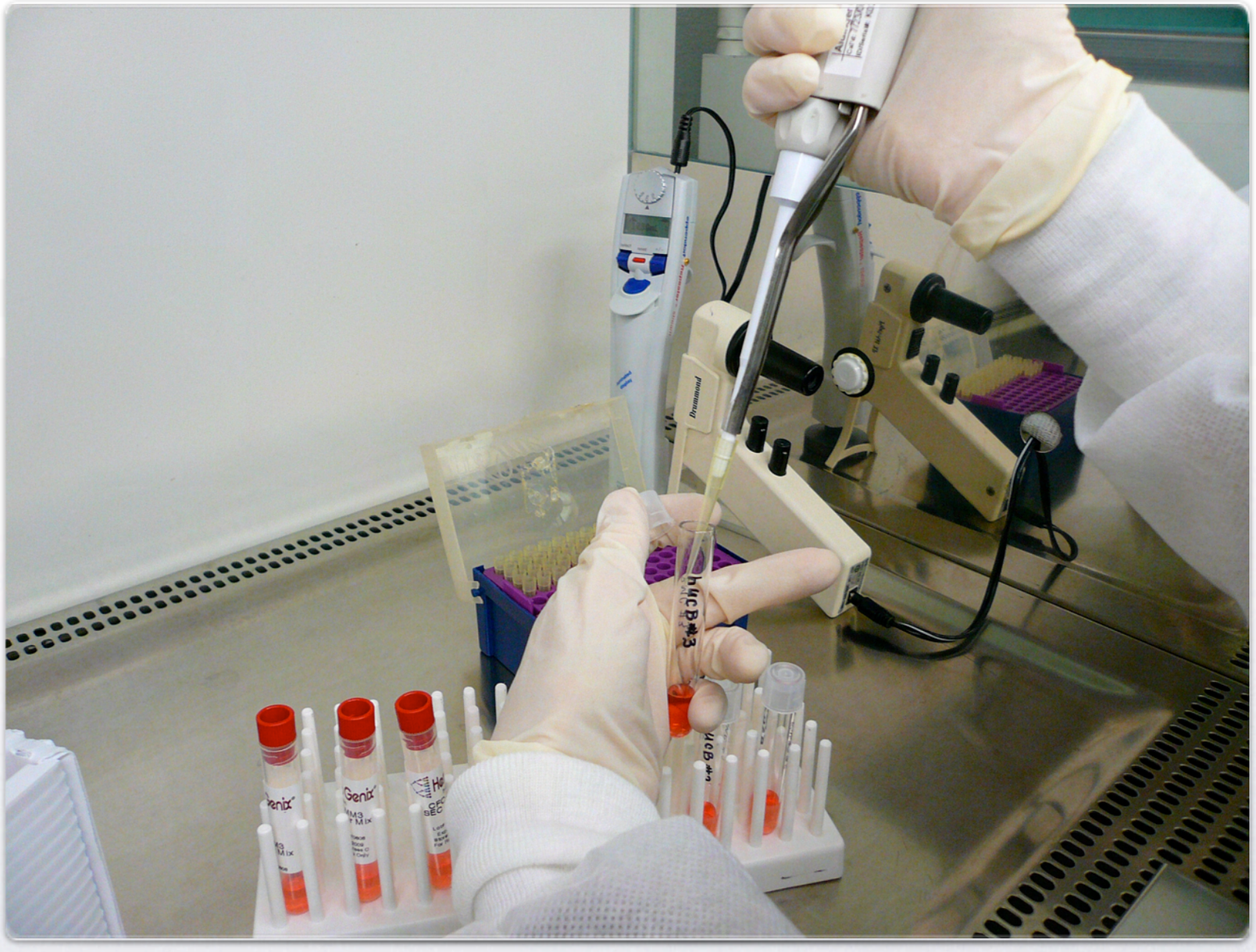
Stem and Progenitor Cell - Quality Control

- Remove 0.1ml of the cell suspension.



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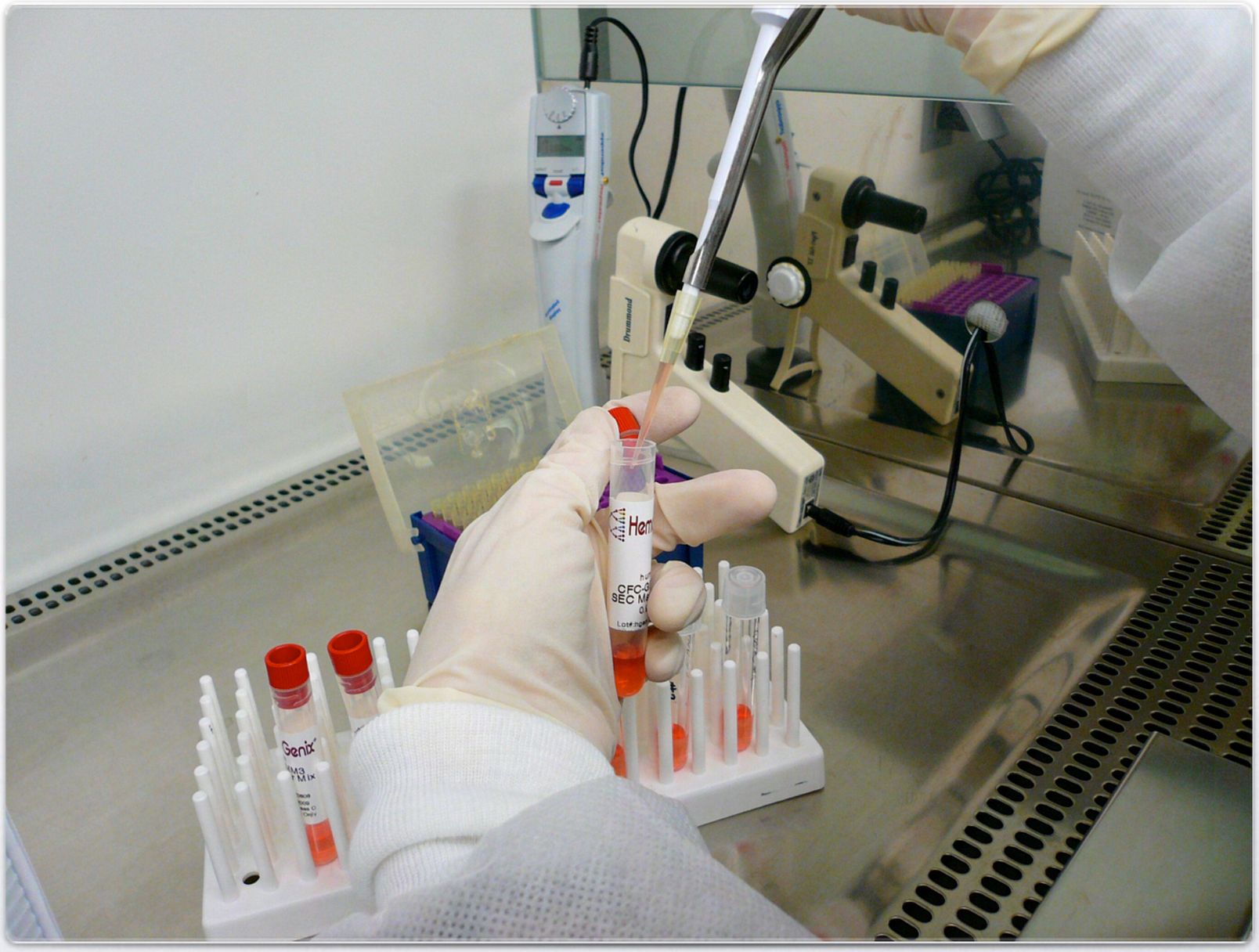
Stem and Progenitor Cell - Quality Control

- Transfer the cell suspension from one sample to one of the HALO® Master Mix tubes containing 0.9ml of the culture reagents.

- The cell concentration is now 10 fold less.



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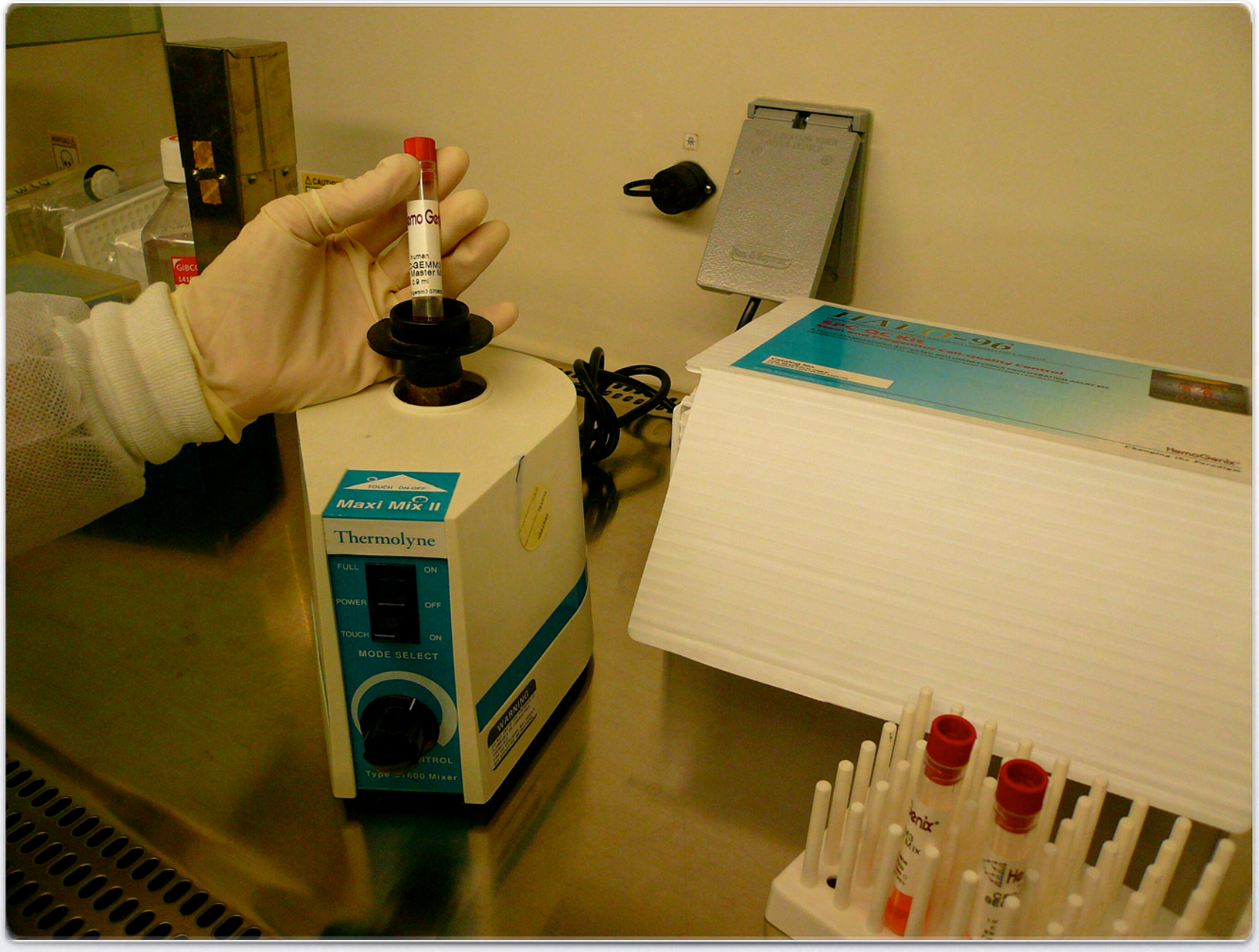
Stem and Progenitor Cell - Quality Control

- Mix the contents of each tube on a vortex mixer.



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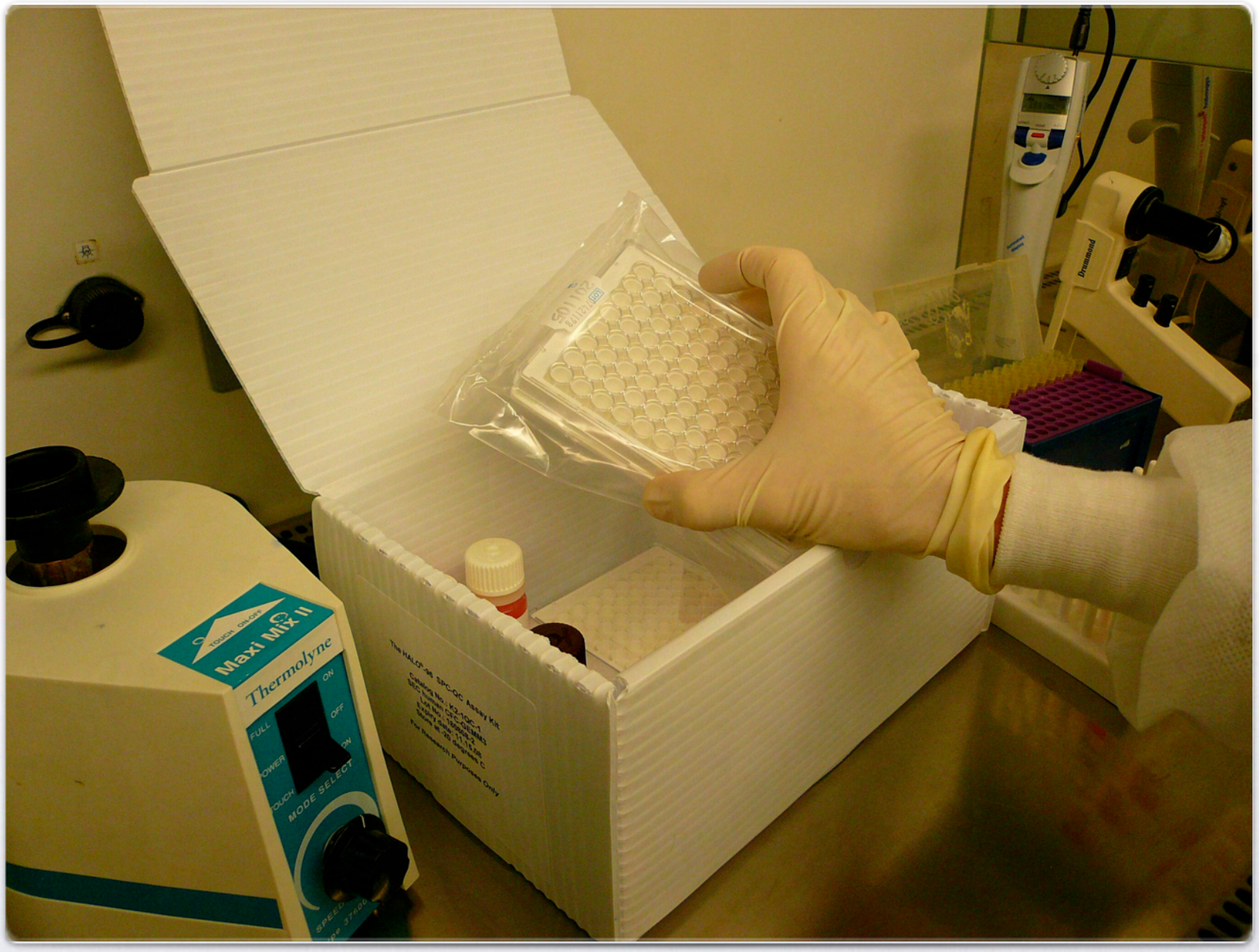
Stem and Progenitor Cell - Quality Control

- Now remove the sterile plate from the kit and take it out of the plastic foil.



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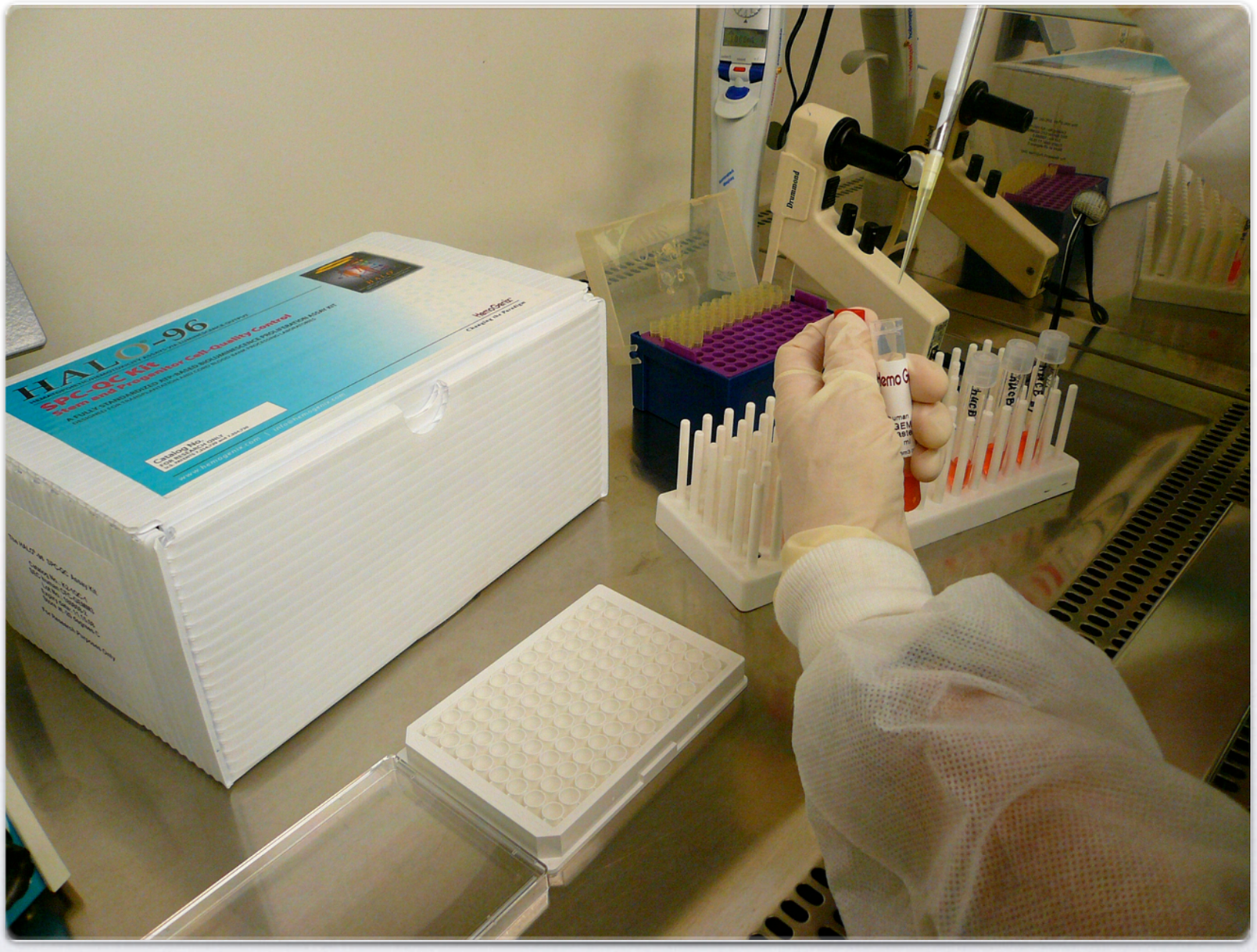
Stem and Progenitor Cell - Quality Control

- Remove 0.1ml of the Culture Master Mix



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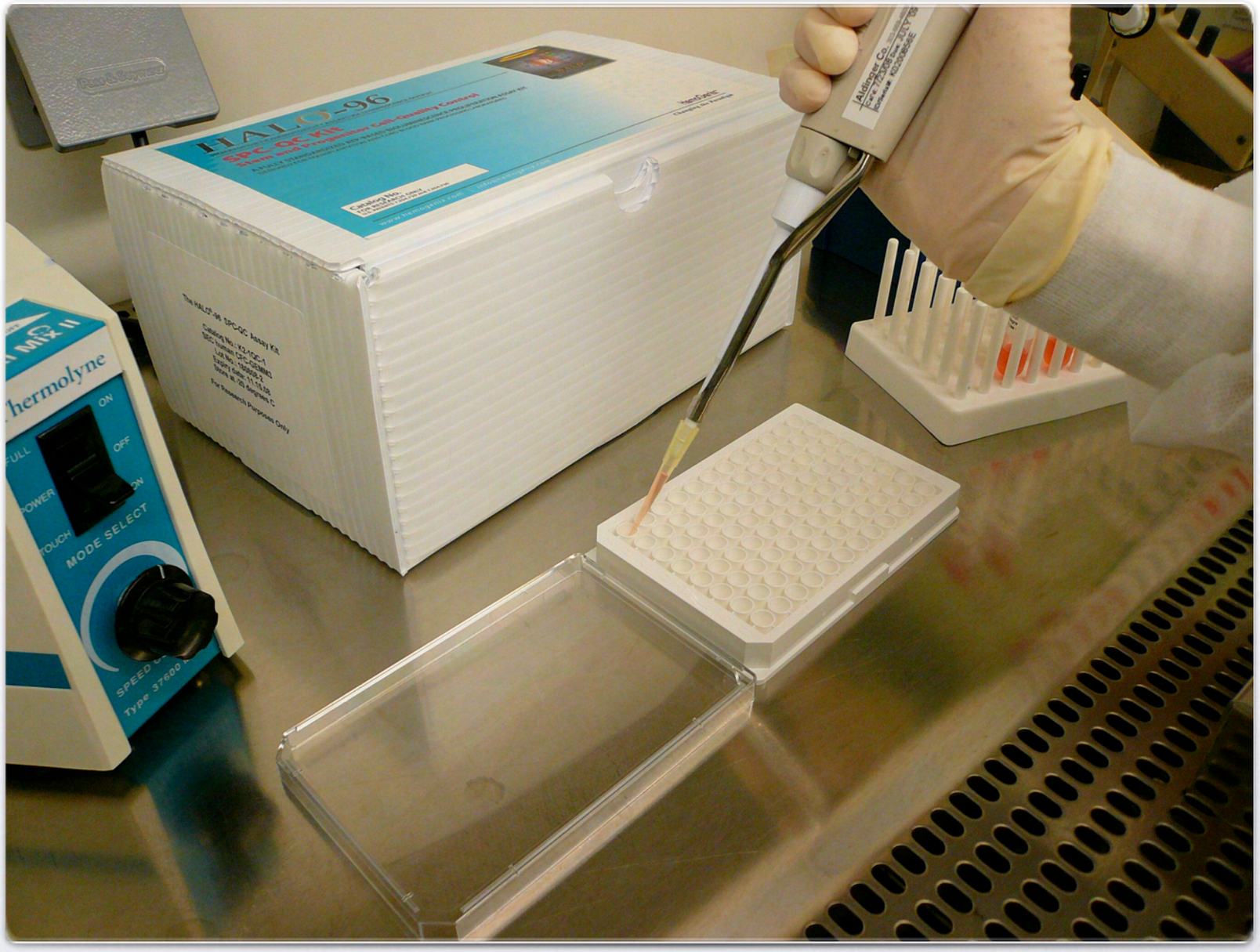
Stem and Progenitor Cell - Quality Control

- Transfer 0.1ml of the Culture Master Mix to the first well of the 96-well plate.

- The cell concentration is now further reduced 10 fold to 5,000 cells/ well.



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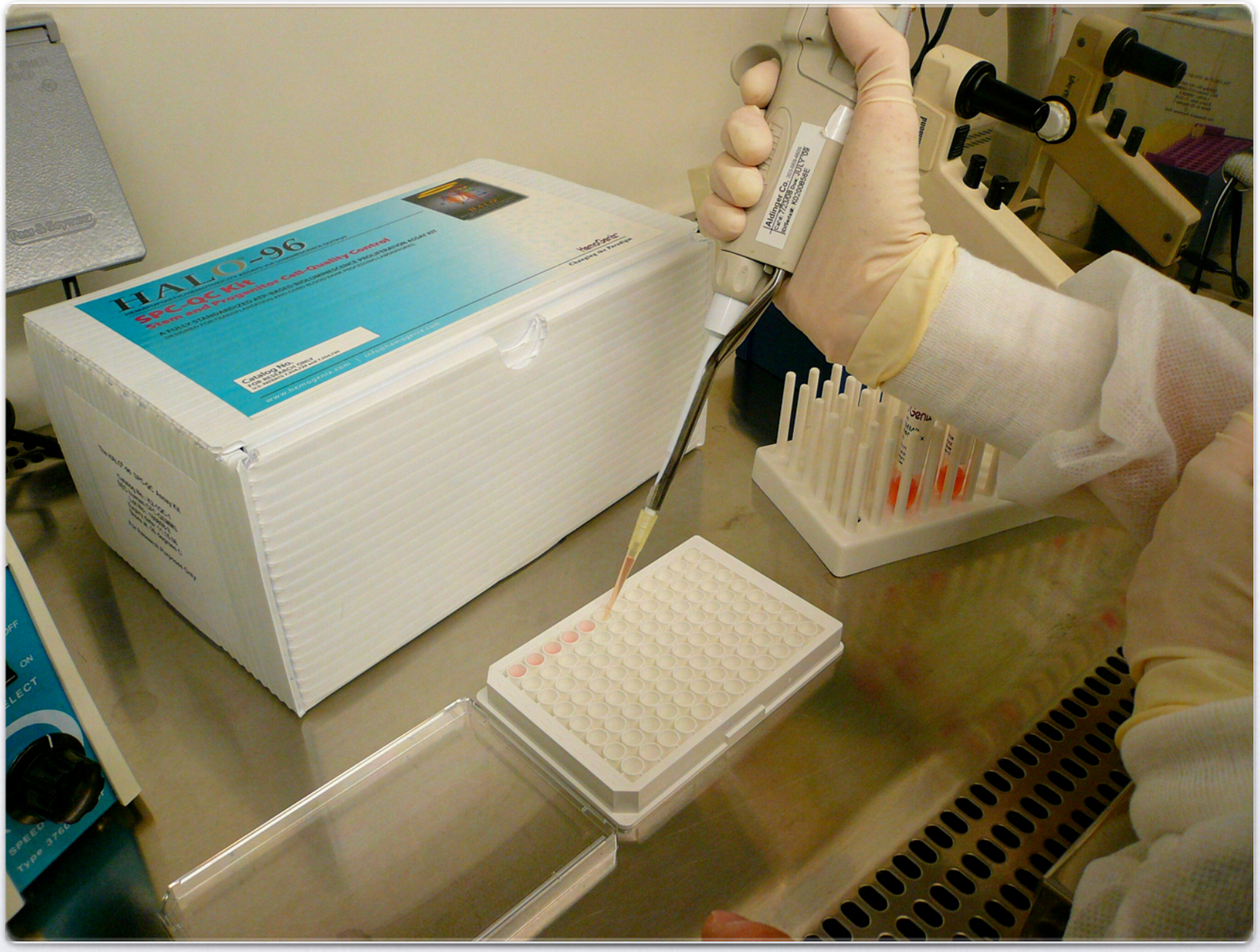
Stem and Progenitor Cell - Quality Control

- Continue filling up 6 replicates of the row with 0.1ml of Culture Master Mix.



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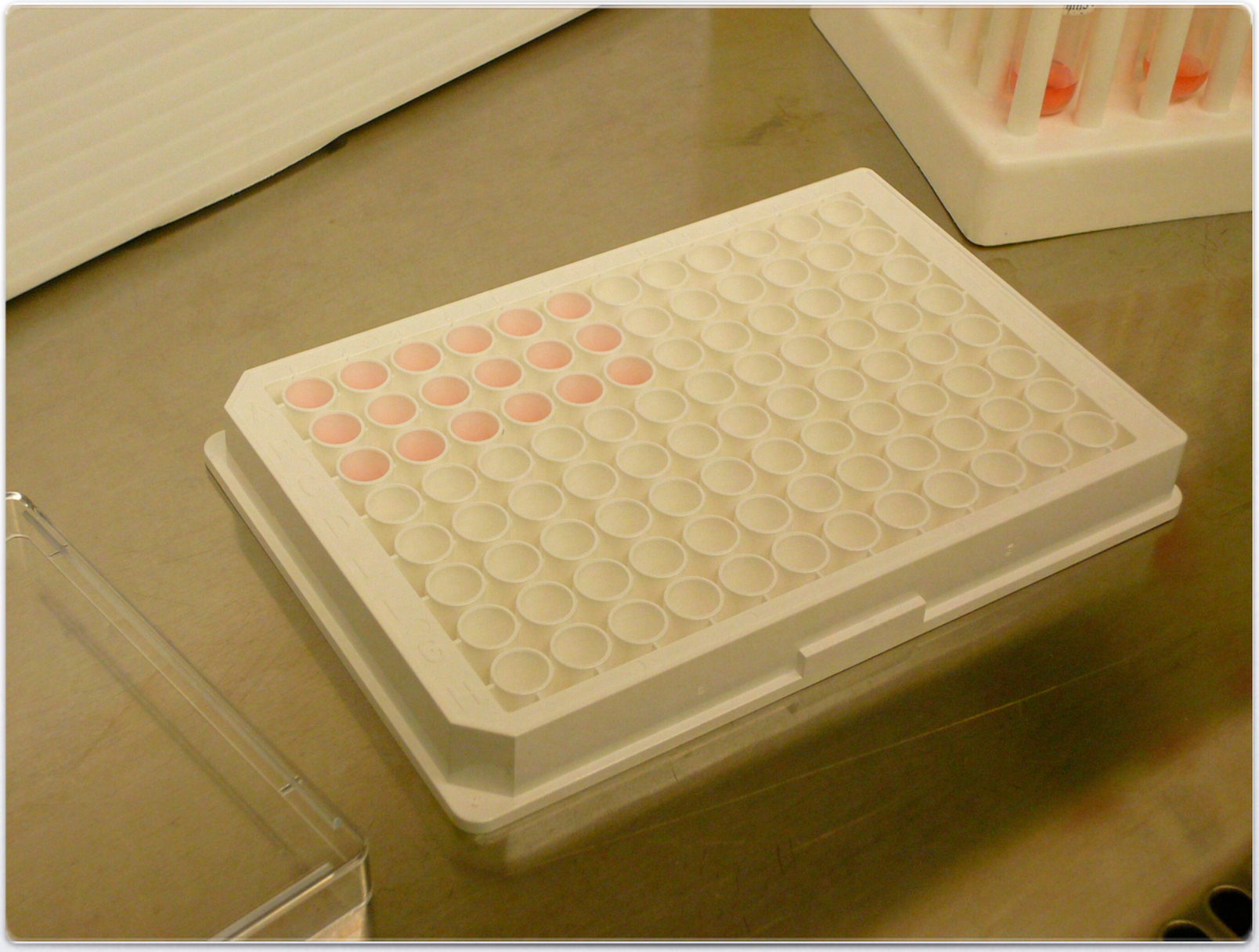
Stem and Progenitor Cell - Quality Control

- Repeat this process for each individual sample.
- For the 3 samples we are testing, there will be 3 rows each with 6 x 0.1ml replicates.



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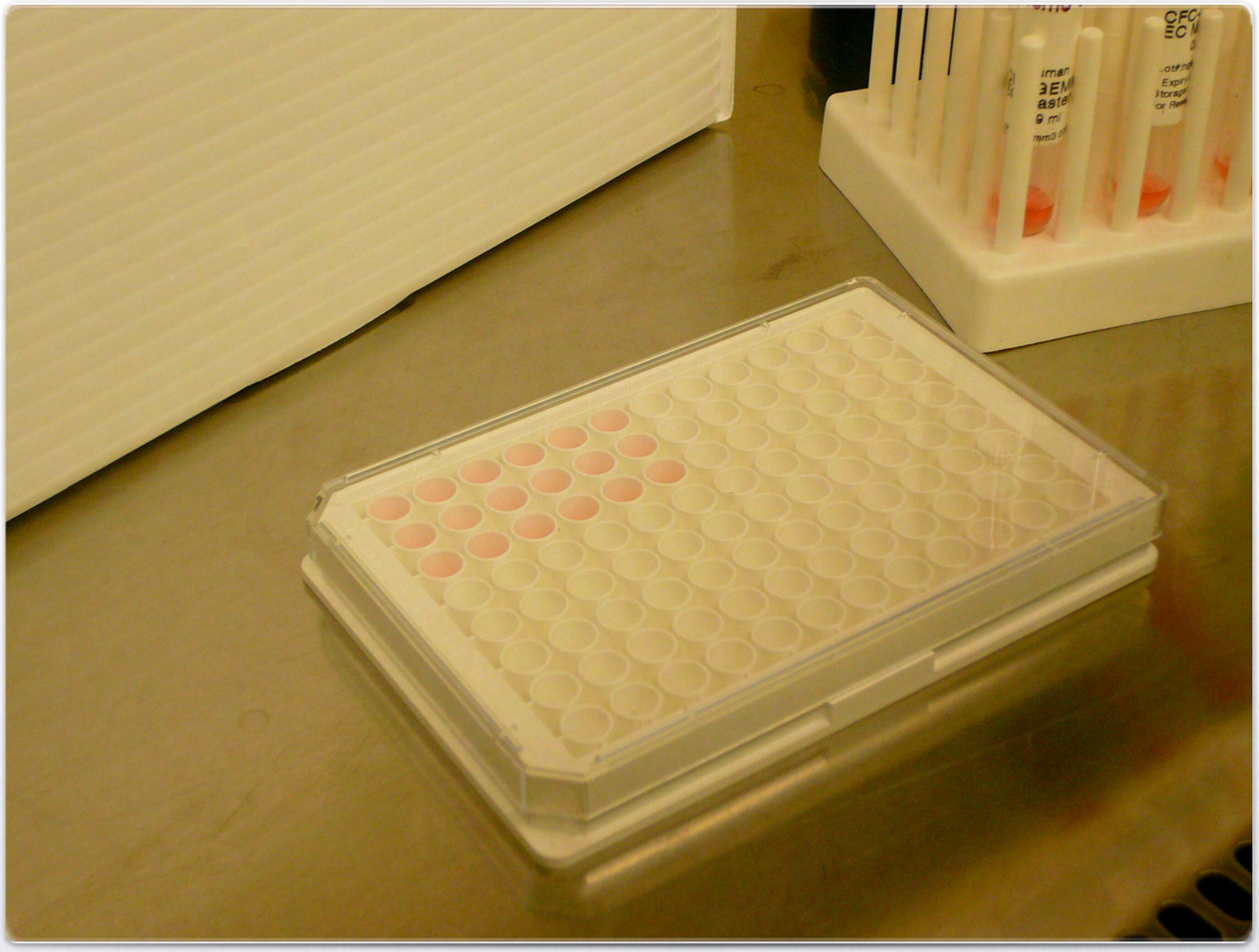
Stem and Progenitor Cell - Quality Control

- Replace the lid



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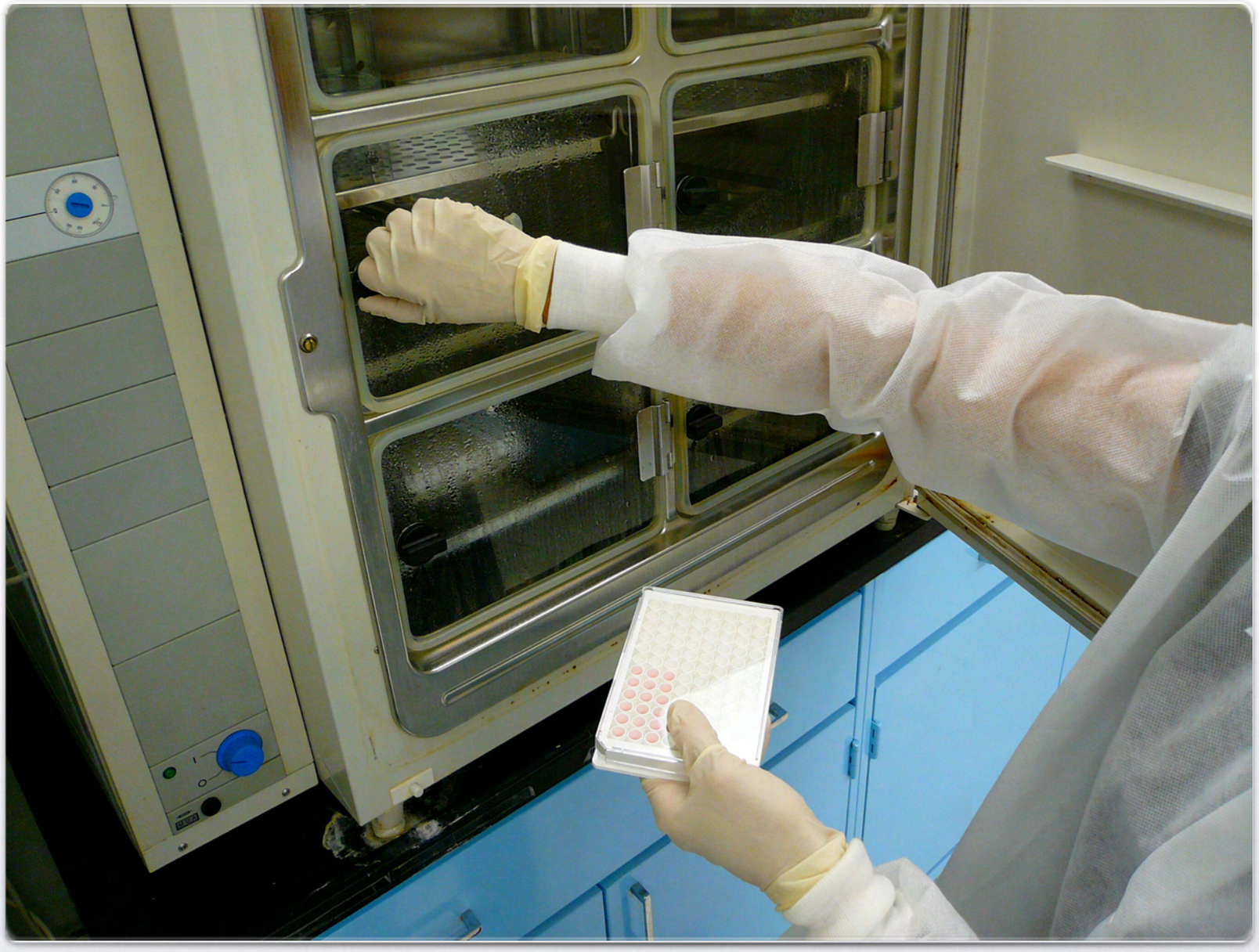
Stem and Progenitor Cell - Quality Control

- Transfer the 96-well plate to an incubator.



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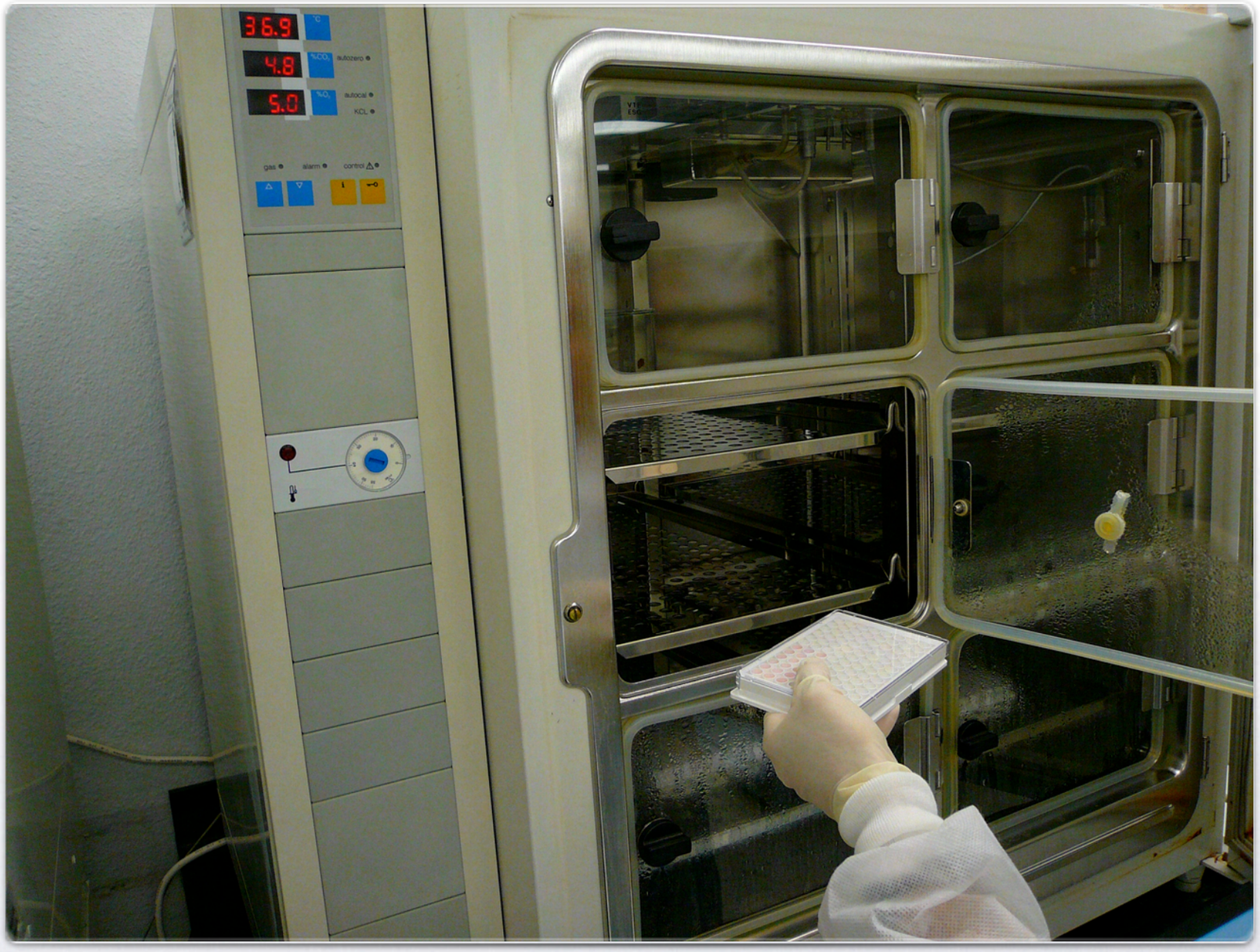
Stem and Progenitor Cell - Quality Control

- Incubate the plate for 5 days at 37ºC in a fully humidified atmosphere containing 5% CO_2 and, if possible, 5% O_2.



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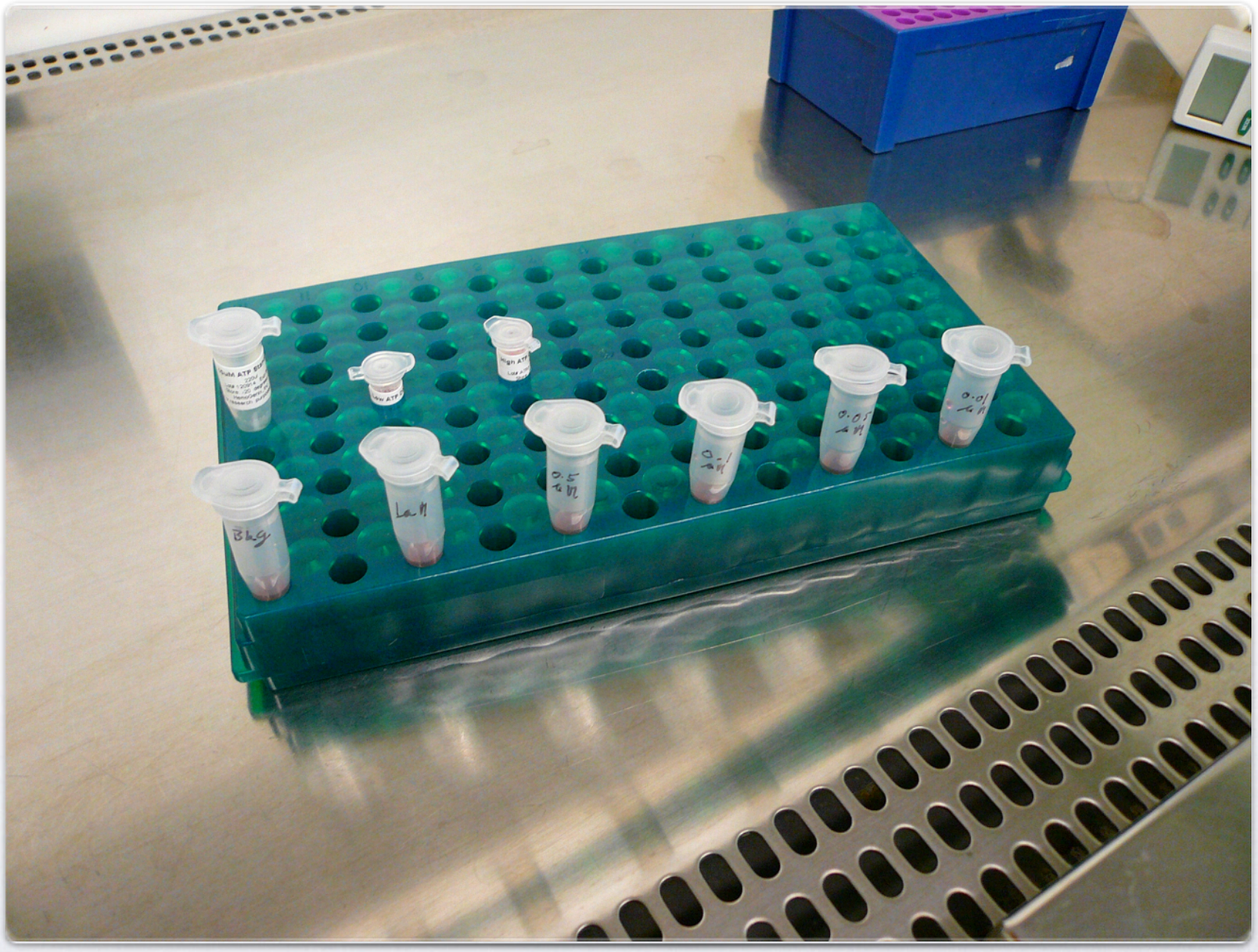
Stem and Progenitor Cell - Quality Control

- After 5 days incubation, we now prepare to measure ATP in the samples.
- First, we have to perform the ATP standard curve.



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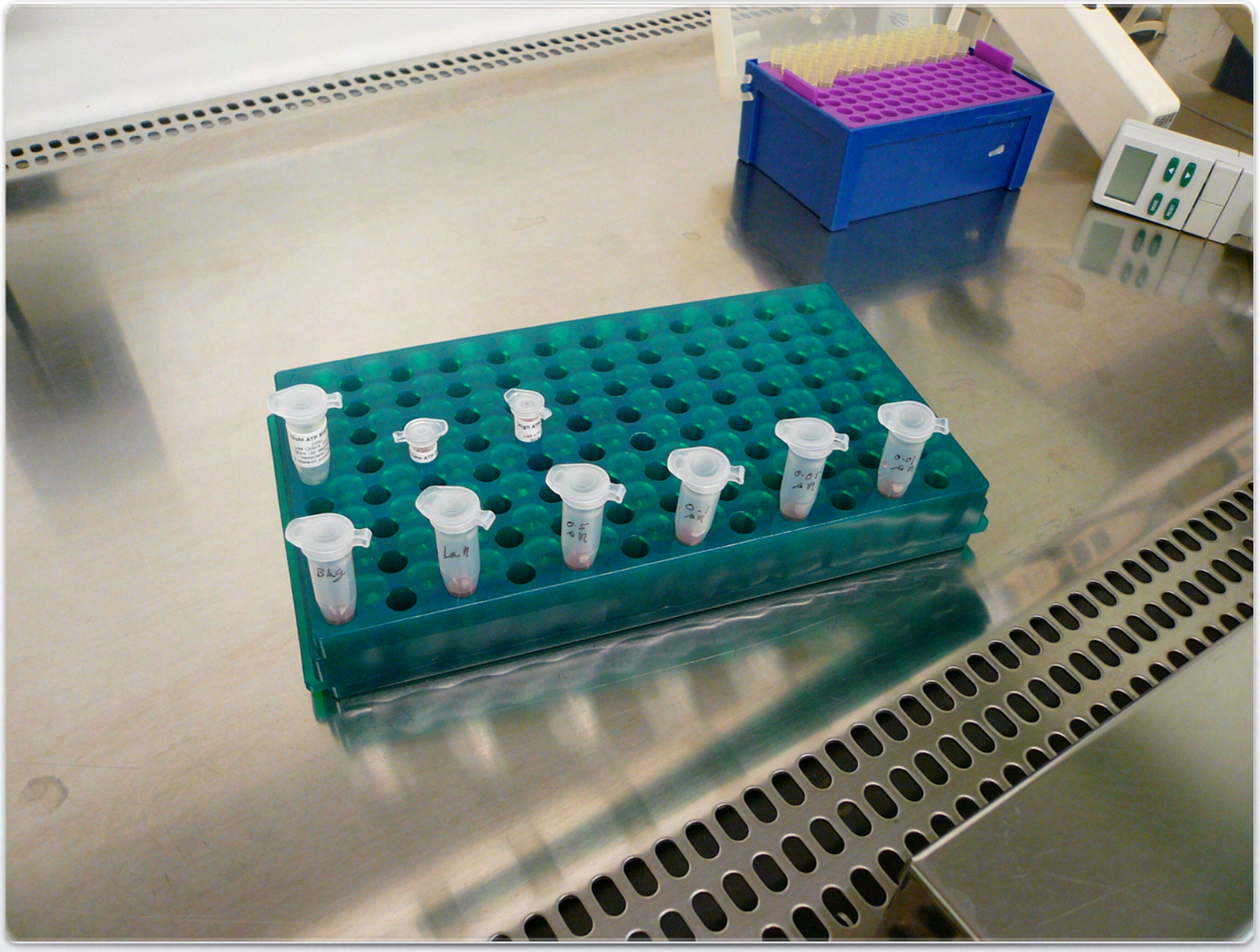
Stem and Progenitor Cell - Quality Control

- An ATP standard and high and low controls are included with the kit.
- Label 5 tubes: 1, 0.5, 0.1, 0.05 and 0.01μM.



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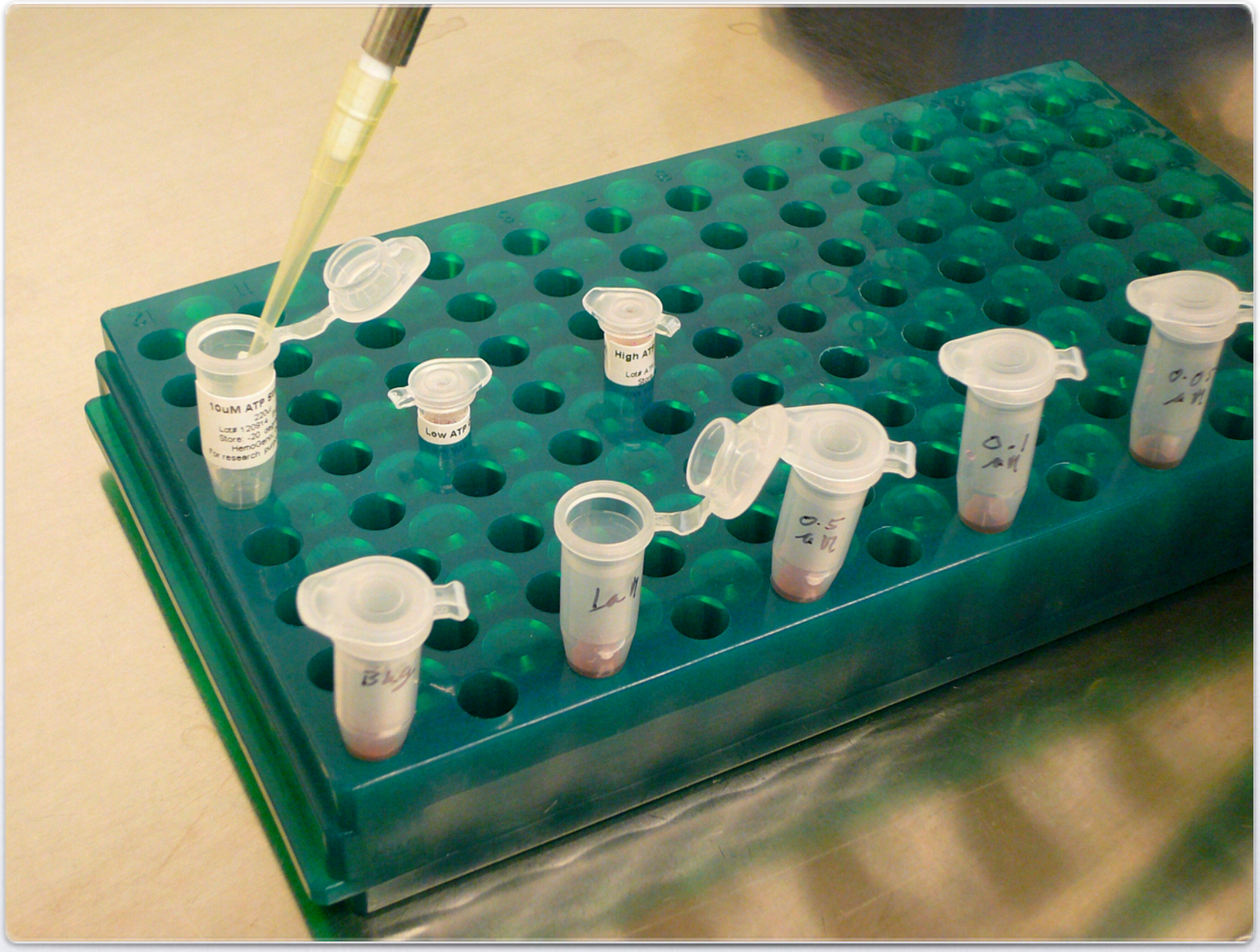
Stem and Progenitor Cell - Quality Control

- Each ATP concentration is prepared by serial dilution: from 1 to 0.1 and then to 0.01μM; from 0.5 to 0.05μM using the medium provided with the kit.



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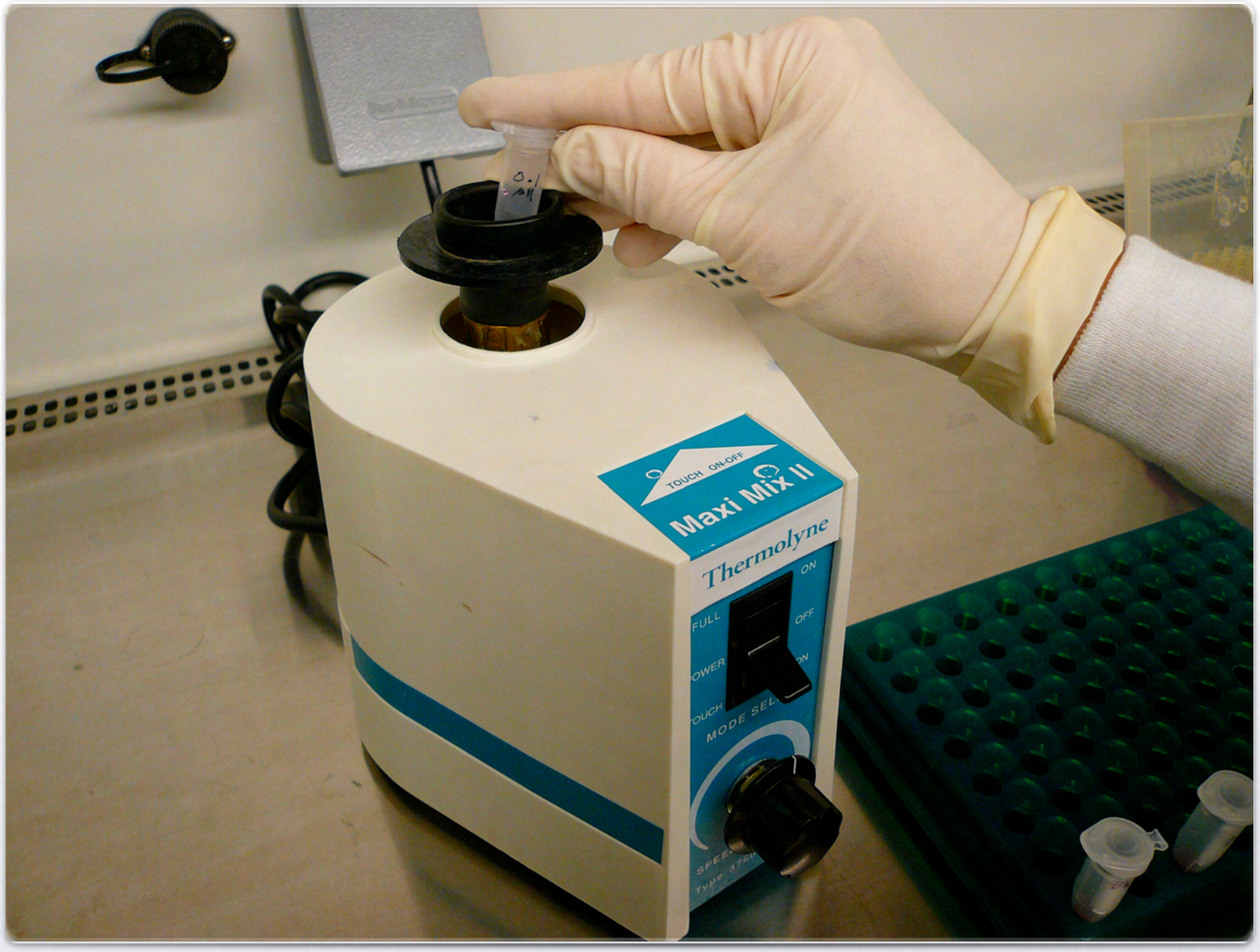
Stem and Progenitor Cell - Quality Control

- Mix the contents of each tube prior to making the next ATP dilution.



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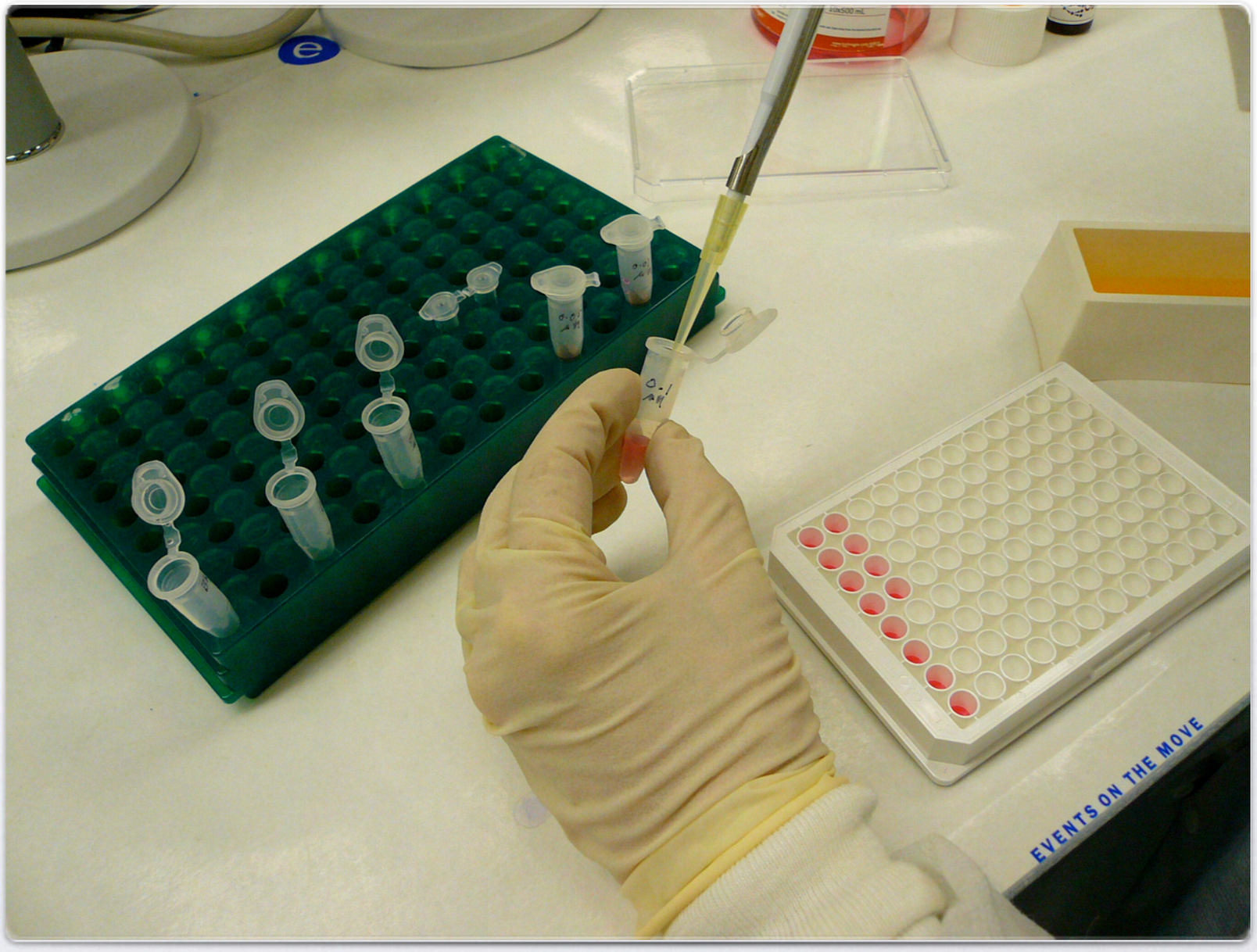
Stem and Progenitor Cell - Quality Control

- Using the non-sterile, 96-well plate provided, first transfer 0.1ml of medium to the first 4 wells in a column.

- Then transfer 0.1ml of each ATP dose to the next 4 vertical wells.



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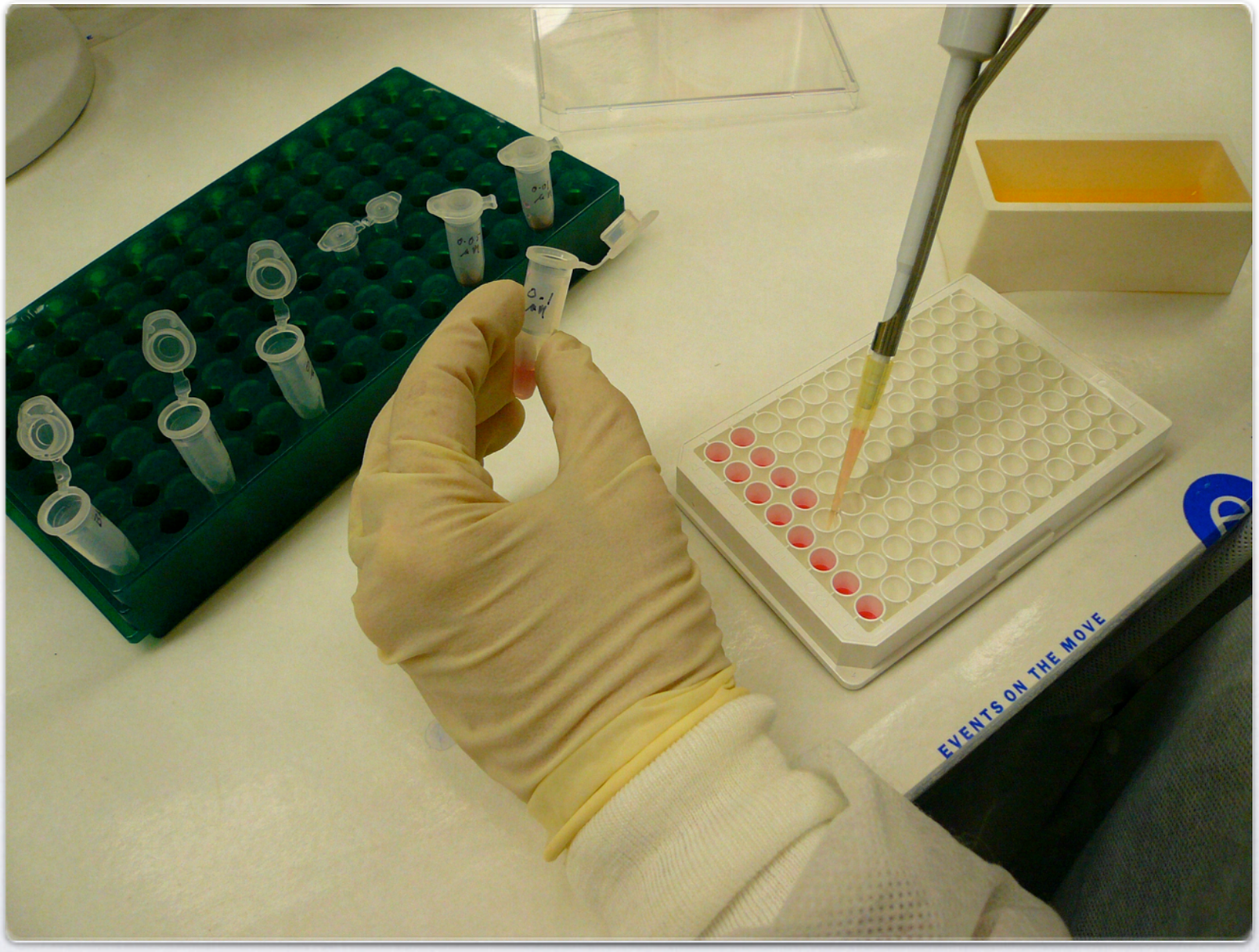
Stem and Progenitor Cell - Quality Control

- Start with the lowest ATP dose after the wells containing the medium.
- The medium is the background.



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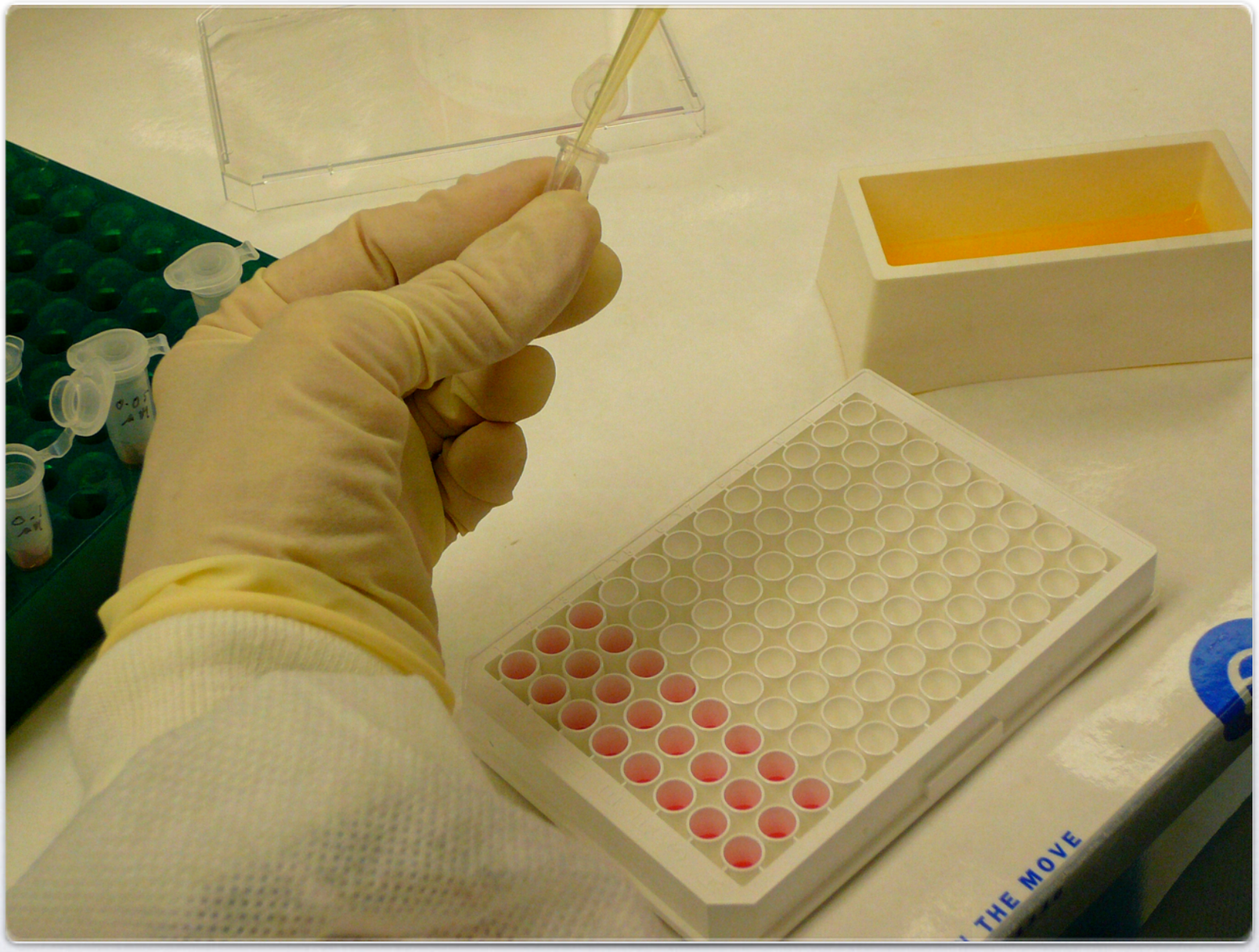
Stem and Progenitor Cell - Quality Control

- The background (medium alone) and all 5 ATP doses have now been transferred to the non-sterile, 96-well plate.



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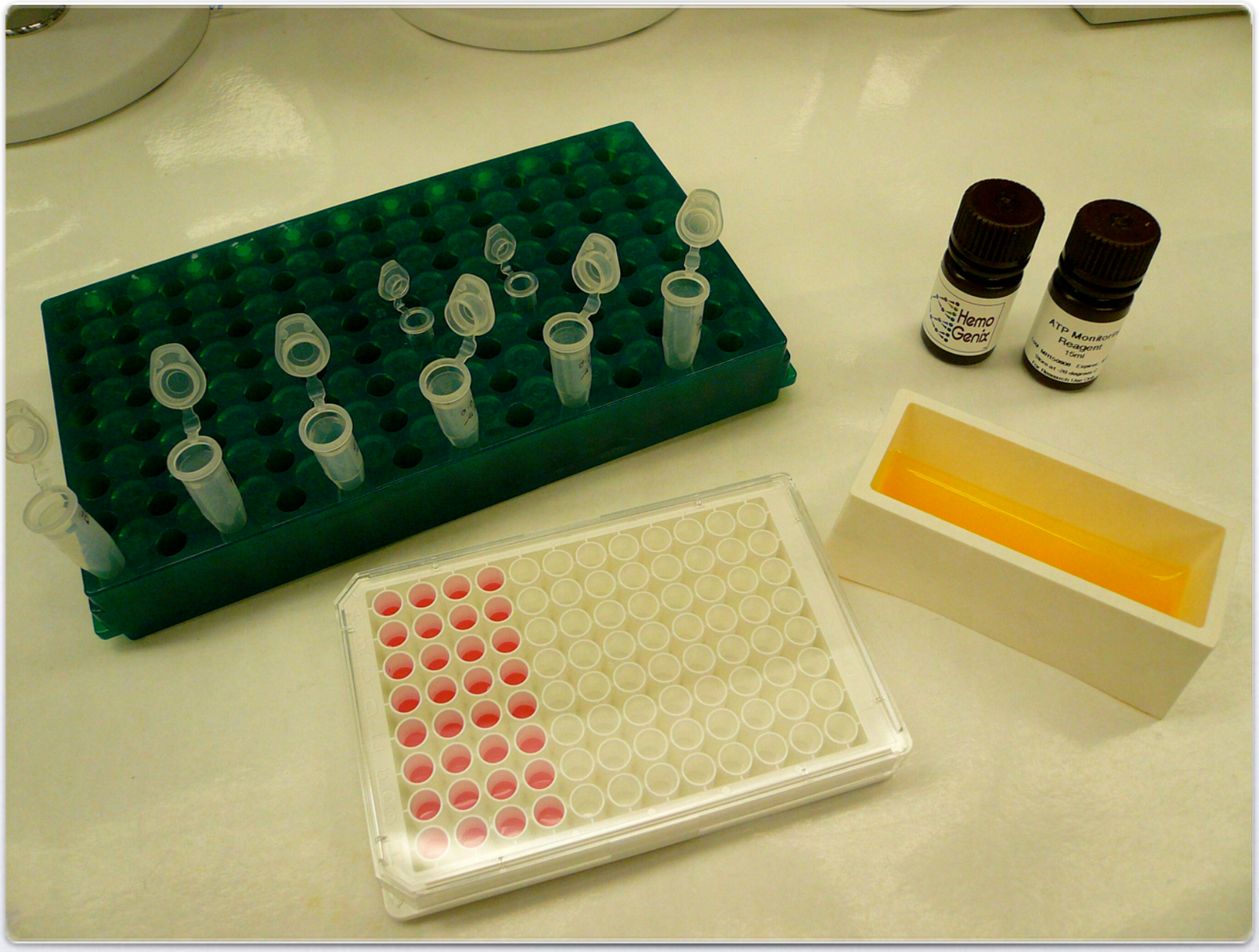
Stem and Progenitor Cell - Quality Control

- Now transfer 0.1ml of the low control to each of 4 replicate wells in the last column.

- Finish by repeating the procedure for the high control.



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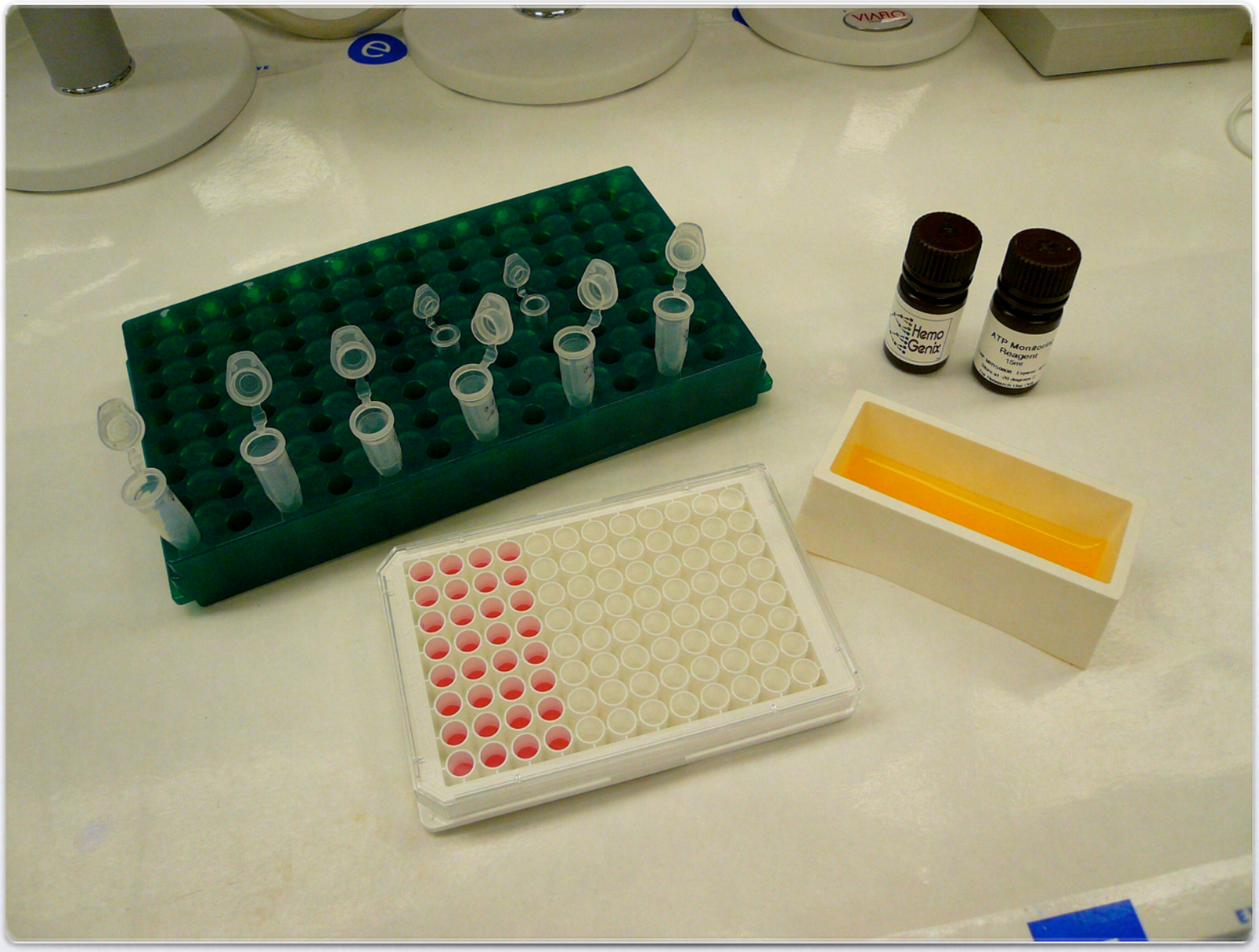
Stem and Progenitor Cell - Quality Control

- The kit contains one or more brown plastic bottles with ATP Monitoring Reagent (ATP-MR).

- This contains a lysis reagent, luciferin and luciferase.



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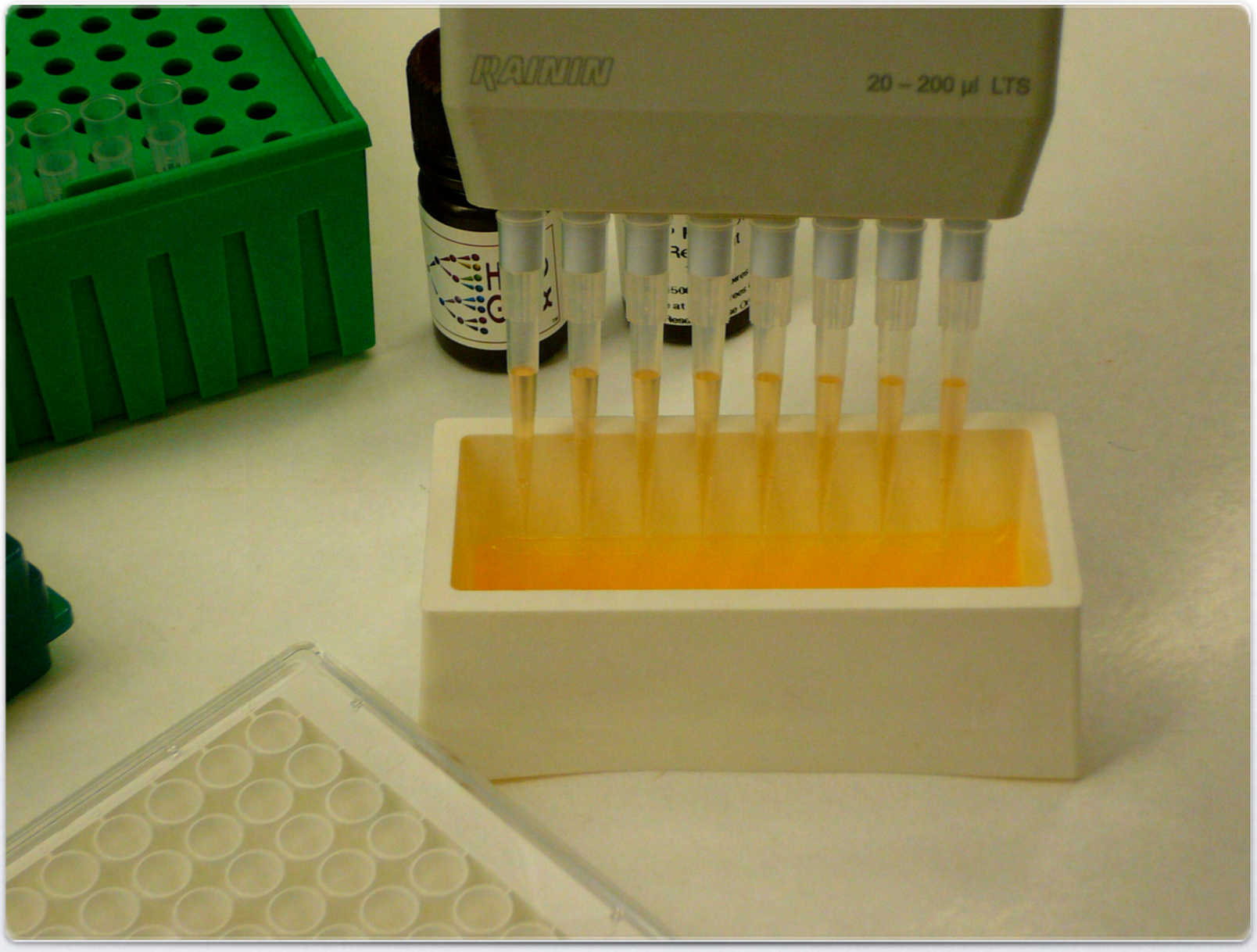
Stem and Progenitor Cell - Quality Control

- Pour the reagent into a reservoir.
- Fix 8 tips to the pipette.
- Set the pipette to pick up 0.1ml of ATP-MR.



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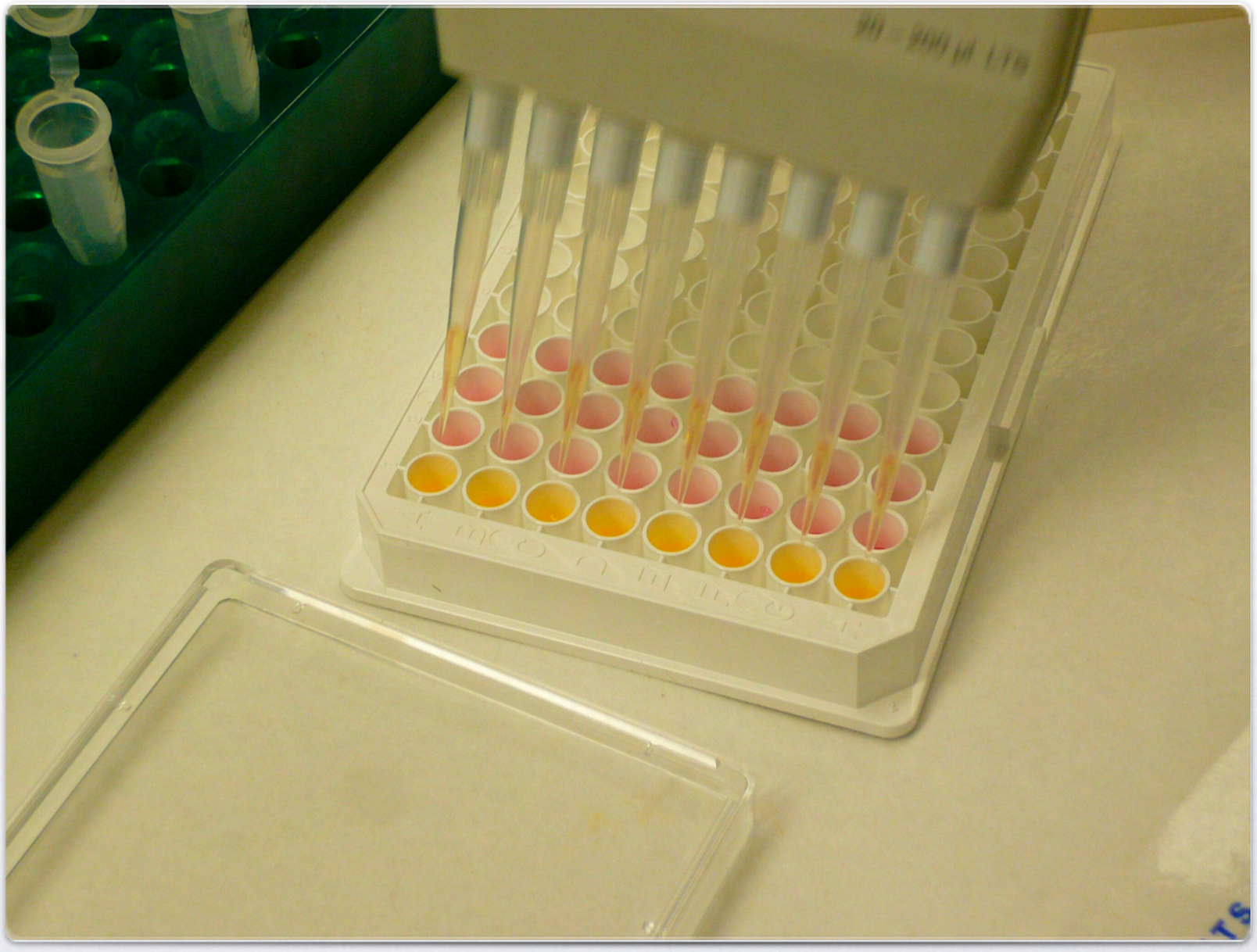
Stem and Progenitor Cell - Quality Control

- Transfer the ATP-MR to the wells of the first column.
- Notice that the color changes when the ATP-MR is added.
- Mix the contents of the wells using the same tips.



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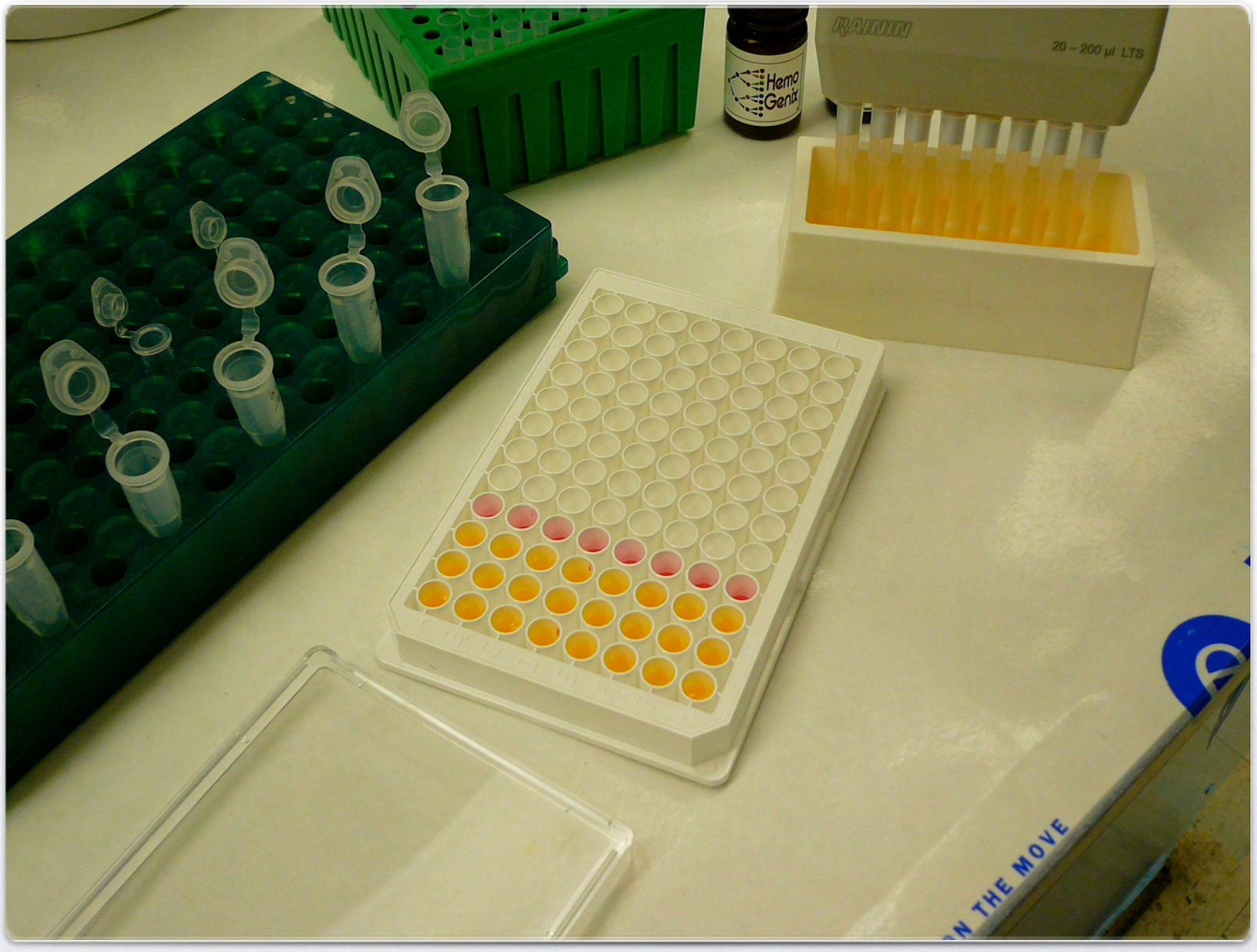
Stem and Progenitor Cell - Quality Control

- Repeat the procedure for each column of well using new tips for each column of replicates.



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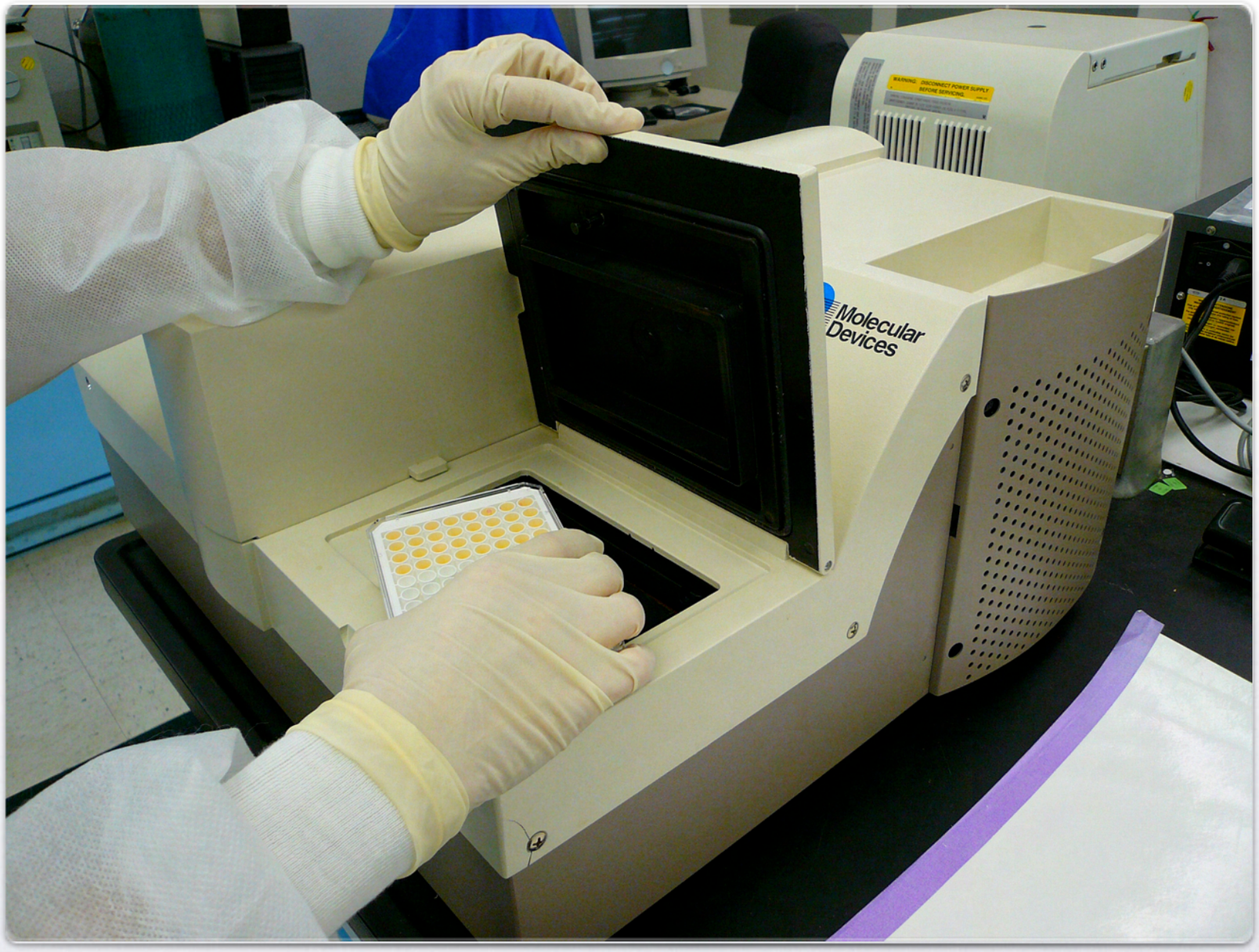
Stem and Progenitor Cell - Quality Control

- Leave the plate to stand for 2 minutes.
- Then transfer the plate to the luminometer.



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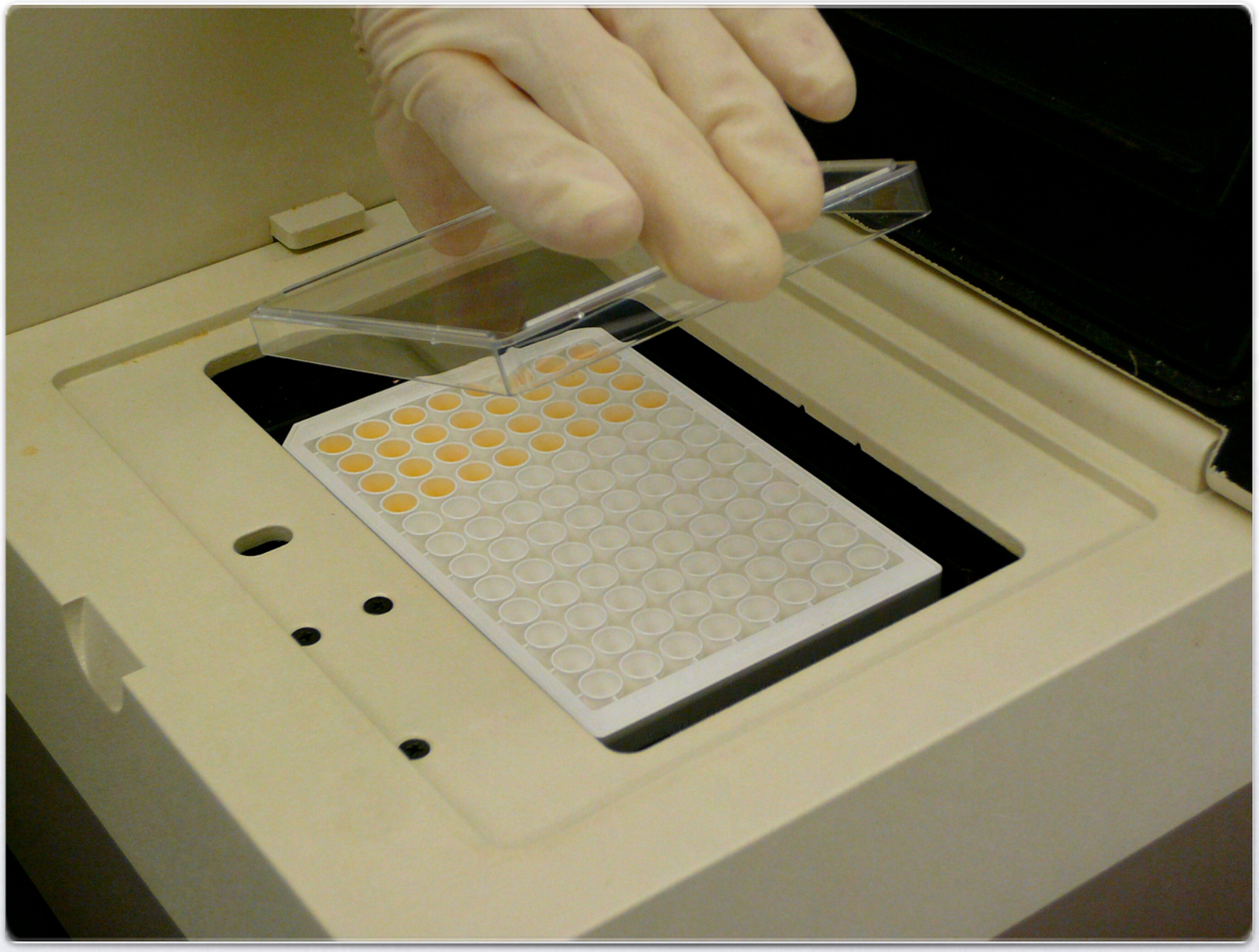
Stem and Progenitor Cell - Quality Control

- Remove the plate lid and close the luminometer.



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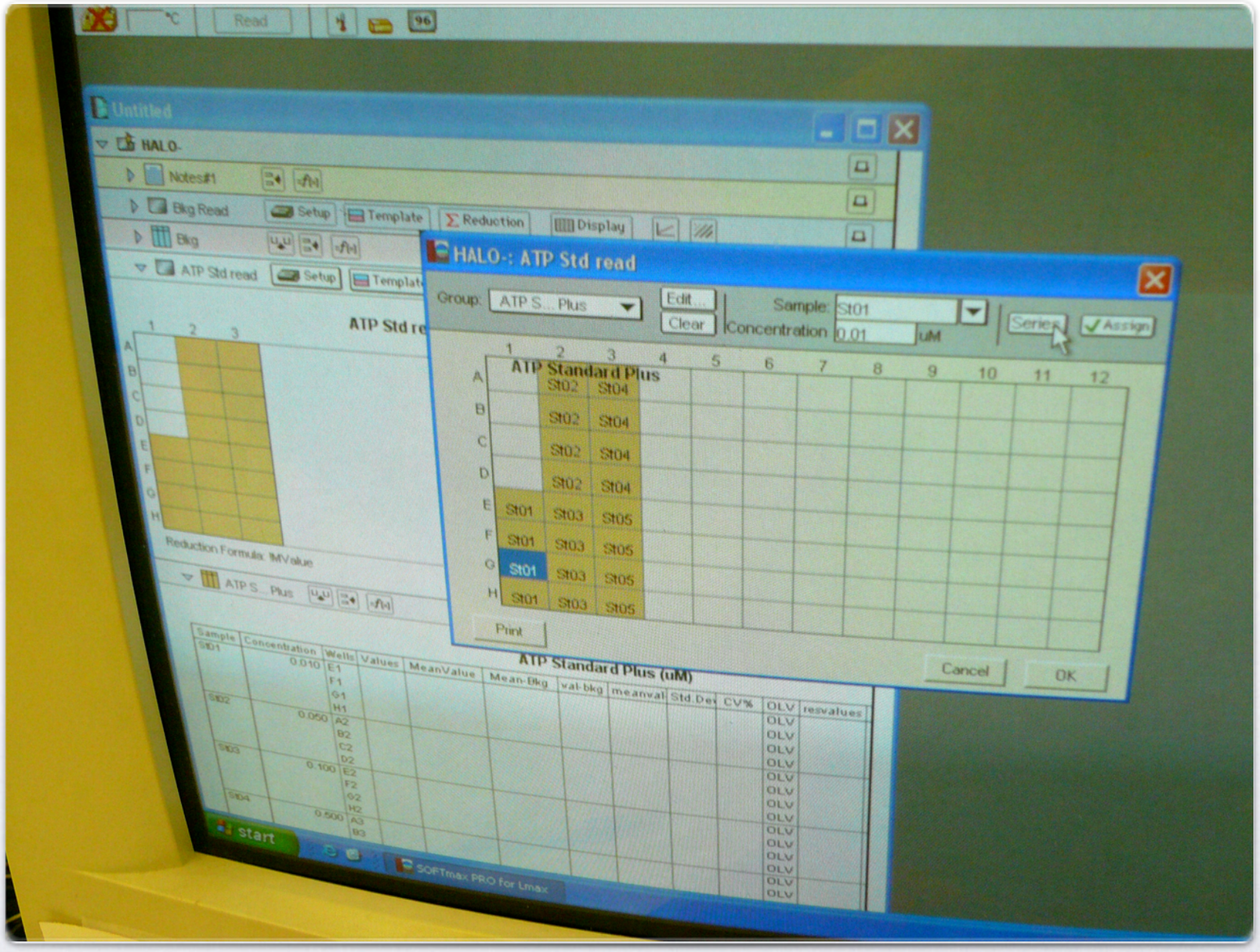
Stem and Progenitor Cell - Quality Control

- Start the ATP standard curve read.
- The luminometer software can be programmed to calculate and plot the ATP standard curve.



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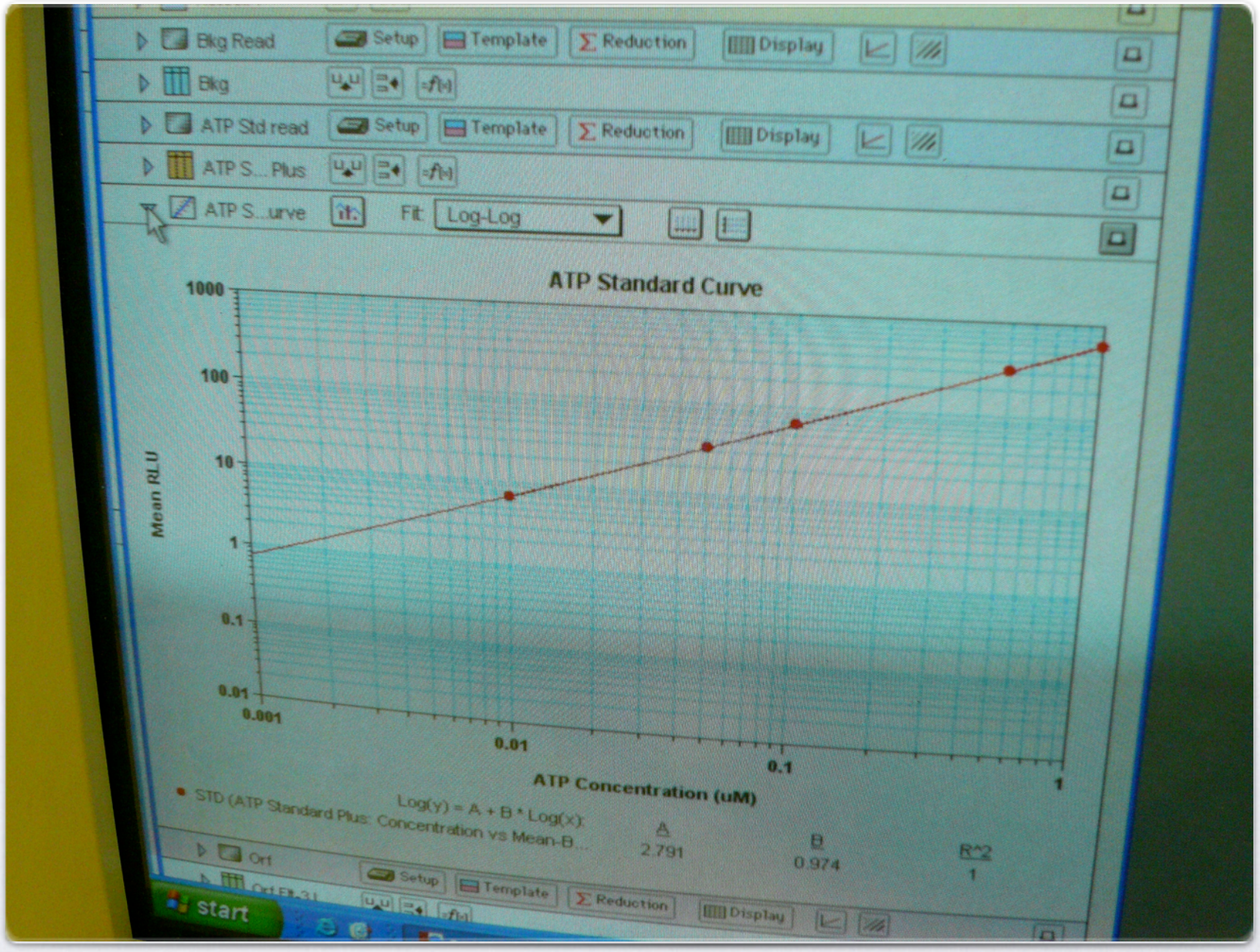
Stem and Progenitor Cell - Quality Control

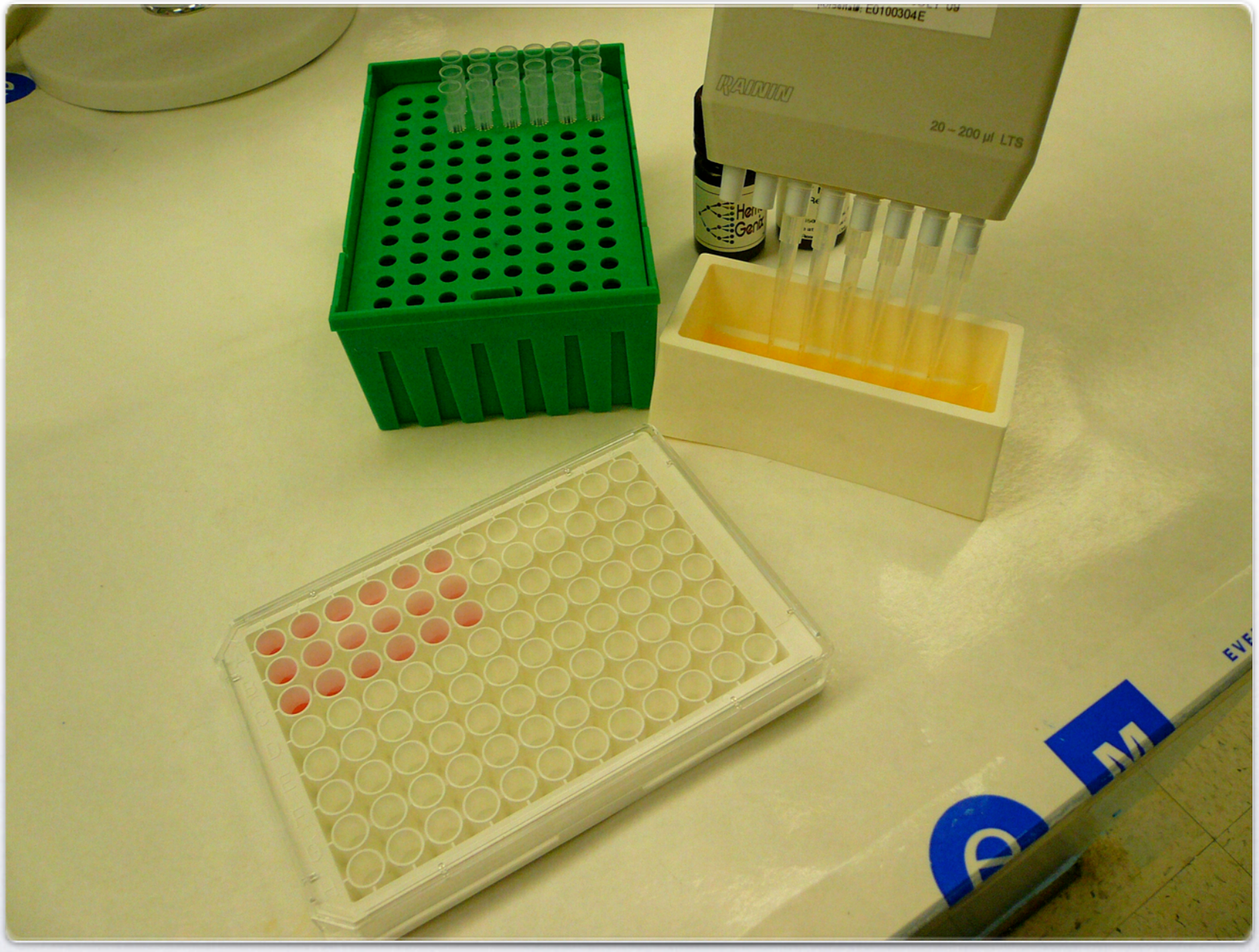
- The ATP standard curve is a linear regression using a log-log scale.
- The goodness of fit (r^2) of the regression should be very near 1.



HALO®-96 SPC-QC

Stem and Progenitor Cell - Quality Control

- Once the ATP standard curve has been performed, remove the sample plate from the incubator.



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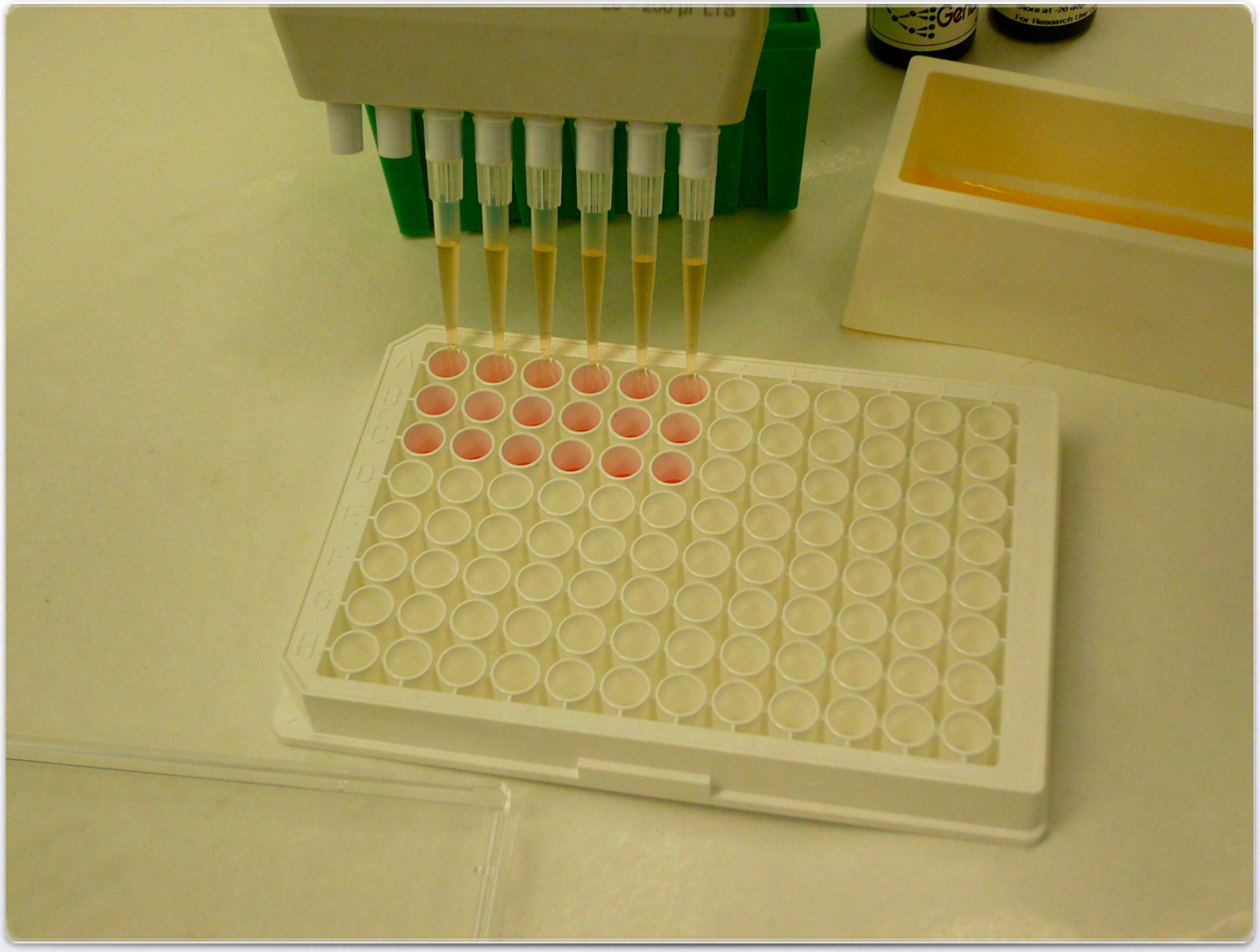
Stem and Progenitor Cell - Quality Control

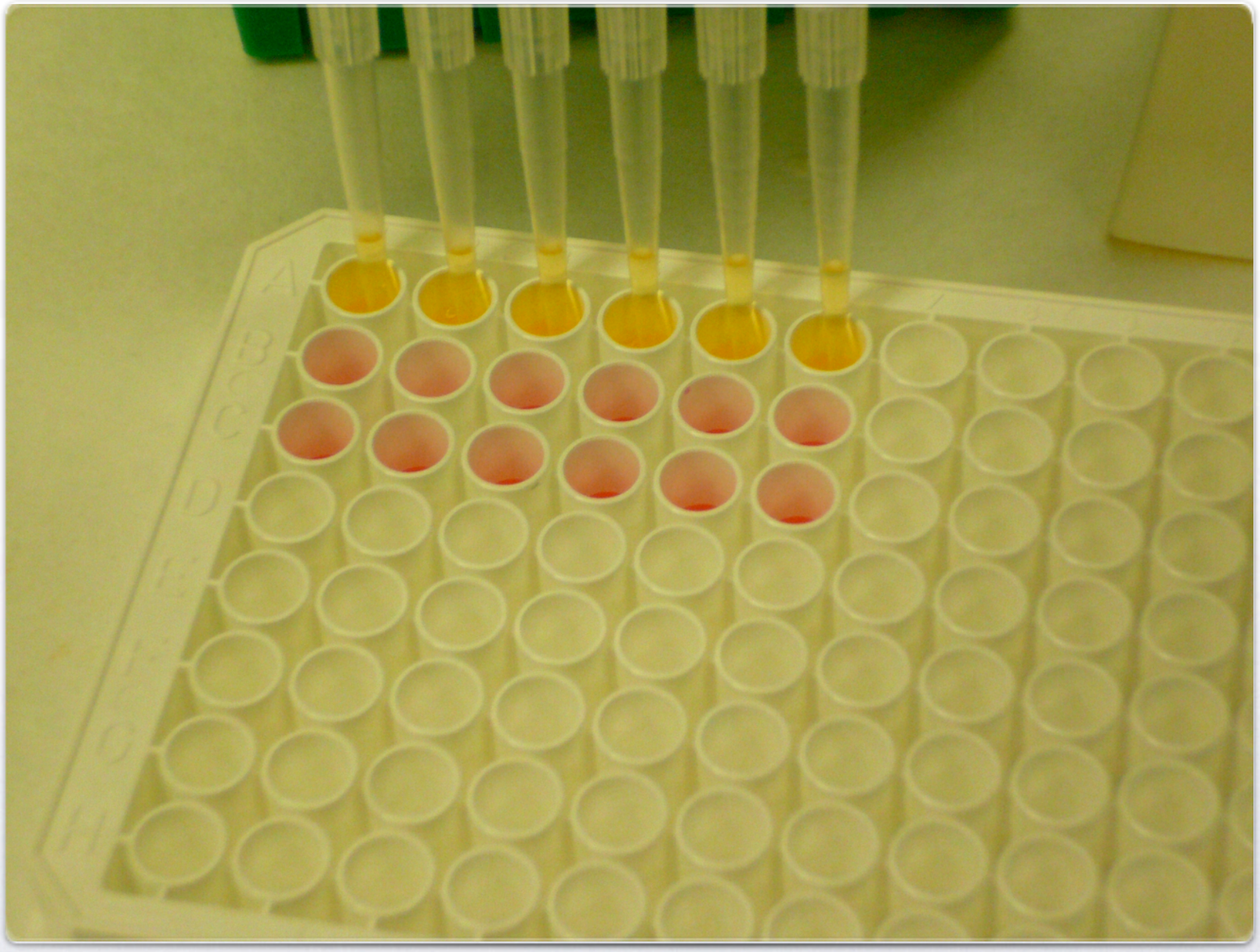
- Using only 6 pipette tips on the multi-channel pipette, add 0.1ml of the ATP-MR to each of the 6 replicate sample wells in the first row.



HALO®-96 SPC-QC

Stem and Progenitor Cell - Quality Control

- After each addition of ATP-MR, mix the contents of each well using the same tips.



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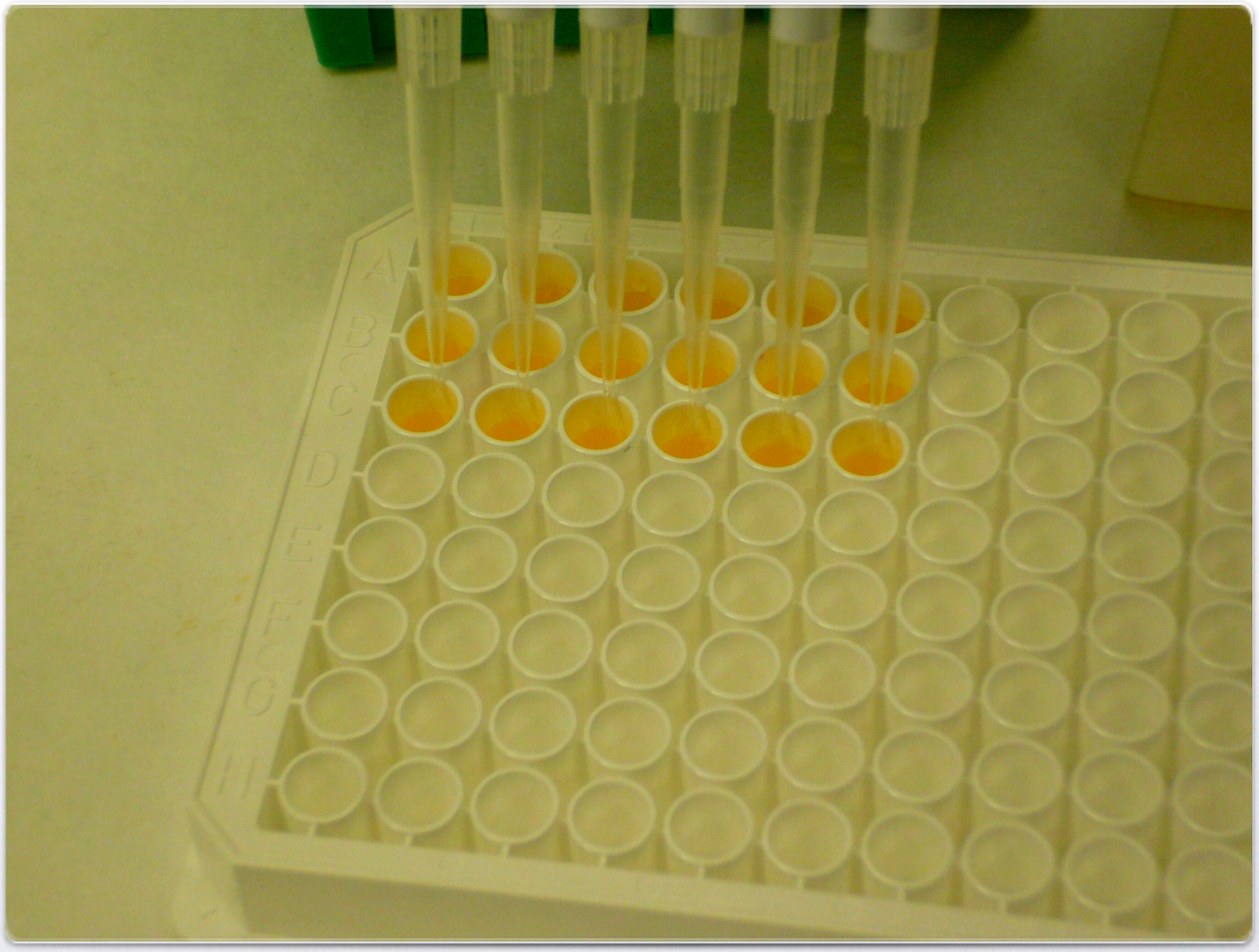
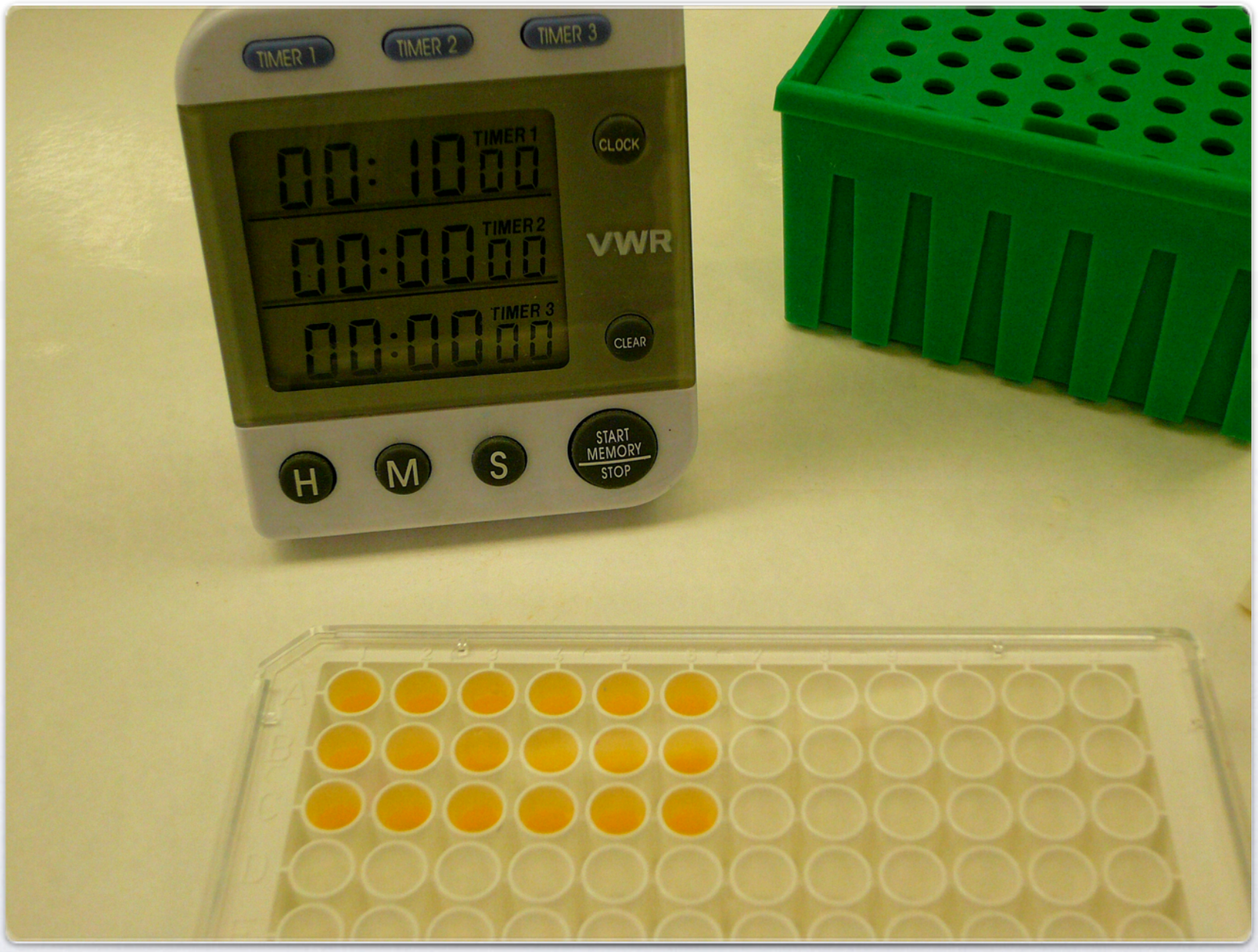
Stem and Progenitor Cell - Quality Control

- Repeat the procedure for each sample, changing the tips each time.



Stem and Progenitor Cell - Quality Control

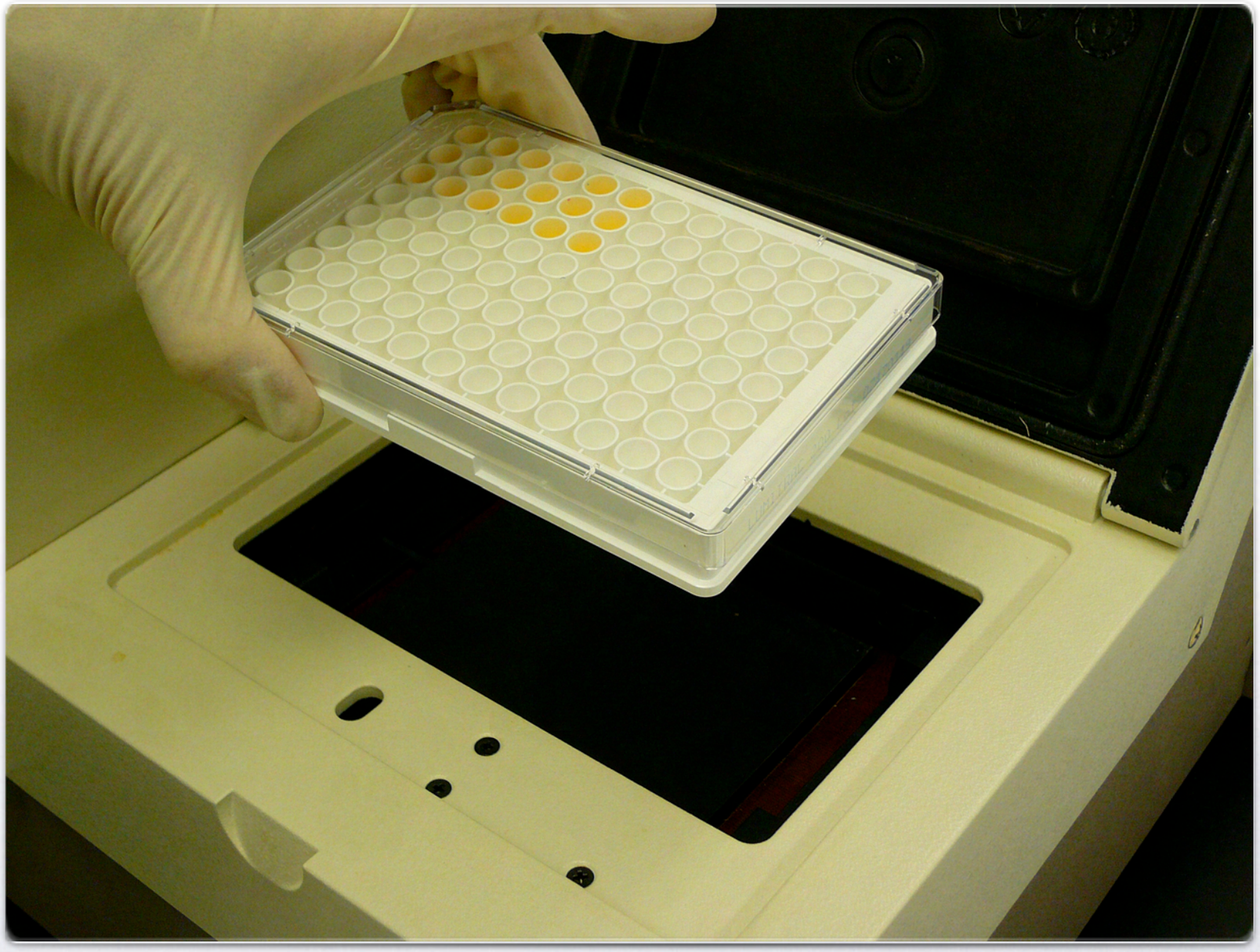
- Cover the plate.
- Incubate the plate at room temperature for 10 minutes.



HALO®-96 SPC-QC

Stem and Progenitor Cell - Quality Control

- After 10 minutes, transfer the plate to the luminometer.
- Remove the lid from the plate.
- Start the instrument to read the luminescence.



The Luminometer Readout

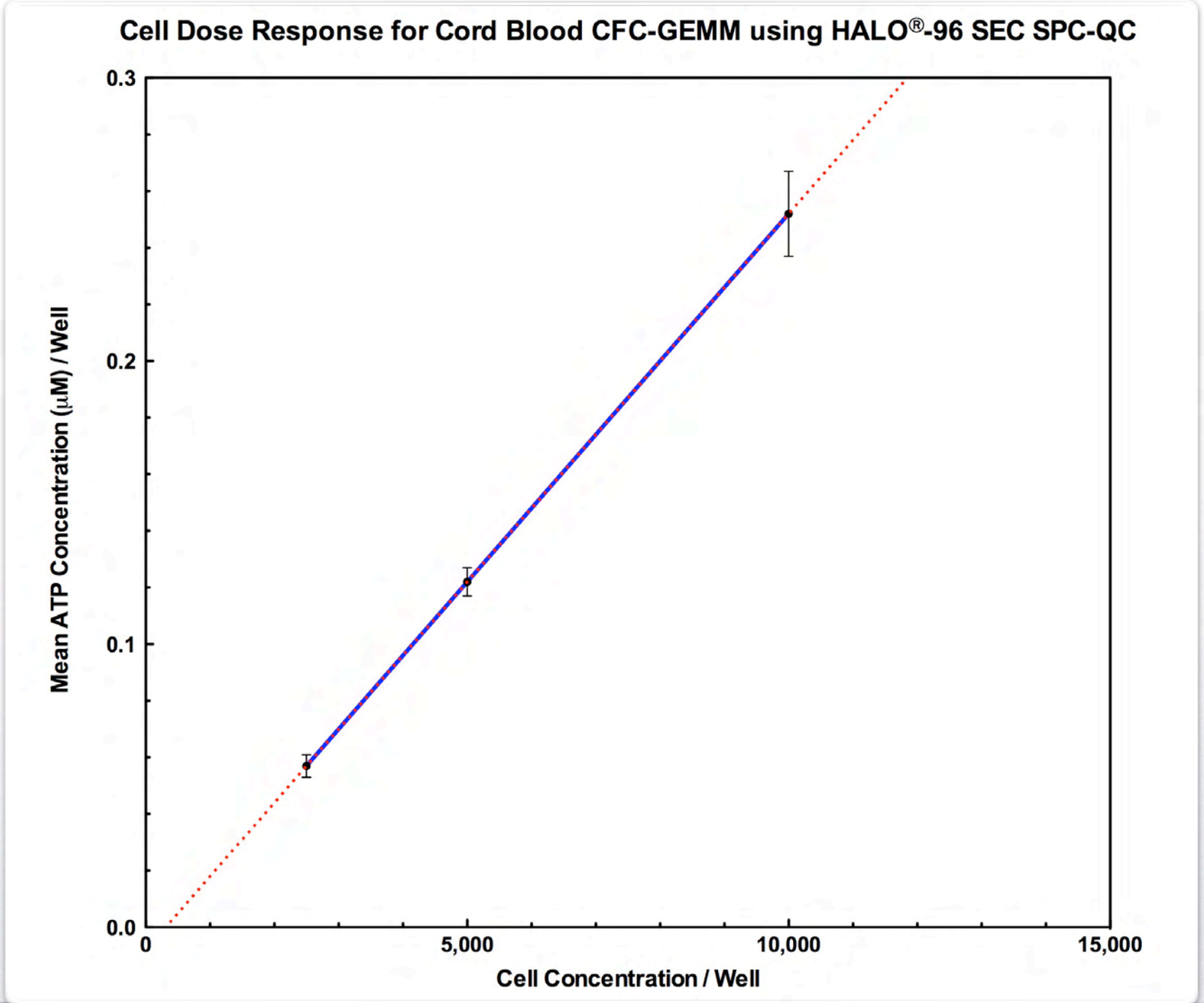
You may be able to customize your luminometer software to give you the output you want. This is an example of an exported SoftMax® file (Molecular Devices). Here you see a cord blood CFC-GEMM cell dose response (2,500, 5,000 and 10,000 cells/well) and the individual RLU values. The software has calculated the mean RLU and the mean RLU minus background (medium alone). It has also calculated the individual RLU values minus the background, followed by the mean standard deviation and percent coefficient of variation. From the ATP standard curve, it has then automatically calculated the individual ATP values and the mean, standard deviation and percent coefficients of variation. Alternatively, your luminometer software may already be programmed to give you this information.

Sample	Wells	Concentration	RLU Values	Mean RLU	Mean RLU-Bkg	RLU-Bkg	Mean RLU-Bkg	Std.Dev.	CV%	ATP values	Mean ATP	St. Dev ATP	%CV
201	A7	2500	18.17	18.044	18	18.1	18	1.1	6	0.058	0.057	0.004	6.2
	B7		18.453			18.4				0.059			
	C7		17.913			17.9				0.057			
	D7		16.566			16.5				0.053			
	E7		17.03			17				0.054			
	F7		17.571			17.5				0.056			
	G7		20.157			20.1				0.064			
	H7		18.491			18.5				0.059			
202	A8	5000	35.904	37.542	37.5	35.9	37.5	1.5	4	0.116	0.122	0.005	4.1
	B8		37.029			37				0.12			
	C8		37.157			37.1				0.121			
	D8		40.406			40.4				0.131			
	E8		36.946			36.9				0.12			
	F8		37.519			37.5				0.122			
	G8		36.277			36.3				0.118			
	H8		39.1			39.1				0.127			
203	A9	10000	72.623	76.014	76	72.6	76	4.3	5.7	0.24	0.252	0.015	5.8
	B9		83.959			83.9				0.279			
	C9		79.857			79.8				0.265			
	D9		70.192			70.2				0.232			
	E9		73.97			73.9				0.245			
	F9		76.643			76.6				0.254			
	G9		76.156			76.1				0.252			
	H9		74.713			74.7				0.247			

HALO®-96 SPC-QC

Stem and Progenitor Cell - Quality Control

- These are the type of results you should expect.
- Crisp, clean quantitative data with small coefficients of variation allowing excellent statistical analyses to be performed.



HemoGenix®

HALO®-96 SPC-QC

- It's fast.
- It's reliable.
- It's reproducible.
- It saves time and money.
- It provides meaningful, quantitative results.
- And if you do need help, we are here for you.

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What HALO®-96 SPC-QC Format should you choose?

- You can choose between HALO®-96 MeC (methyl cellulose) or HALO®-96 SEC (Suspension Expansion Culture).
- In contrast to the former, HALO®-96 SEC SPC-QC:
  - Does not involve dispensing methyl cellulose.
  - This produces lower errors and therefore lower CVs.
  - It is faster and easier to perform.
  - It is also 2 x more sensitive than HALO®-96 MeC SPC-QC.

HALO®-96 SPC-QC

Available for 4 applications

- Single stem cell (CFC-GEMM) kit: For routine QC.
- Duel stem cell kit: To detect both primitive (HPP-SP) and mature stem cells (CFC-GEMM).
- 4-Population Kit: To monitor the hematopoietic status of patients (CFC-GEMM, BFU-E, GM-CFC and Mk-CFC)
- 7-Population Kit: To monitor the lympho-hematopoietic status of patients (all of the above plus T-CFC, B-CFC and background).

HemoGenix®

HALO®-96 SPC-QC

Order kits directly from HemoGenix® by:

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(2) Faxing 719-264-6253

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