Lab Notebook 2024

Callum Malcolm

Contents

LN Repository

Rack 1

- Location: Tank 2, Rack 1, Row H (Bottom)

Location	Cap ID	Description	Date
1	Grey	Empty - Marker	-
2	Ramos BC 1	Ramos RTX CDC Baseline	12/06/2024
3	Ramos BC 1	Ramos RTX CDC Baseline	12/06/2024
4	Ramos BC 1	Ramos RTX CDC Baseline	12/06/2024
5	C4 DP2	Ramos RTX CDC C4-DP2	-
6	C5 DP2	Ramos RTX CDC C5-DP2	-
7	C1 DP2	Ramos RTX CDC C1-DP2	-
8	R3 DP2	Ramos RTX CDC R3-DP2	-
9	C6 DP2	Ramos RTX CDC C6-DP2	-
10	C3 DP2	Ramos RTX CDC C3-DP2	-
11	C2 DP2	Ramos RTX CDC C2-DP2	-
12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-
16	-	-	-
17	-	-	-
18	-	-	-
19	-	-	-
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21	-	-	-
22	-	-	-
23	-	-	-
24	-	-	-
25	-	-	-
26	-	-	-
27	-	-	-
28	-	-	-
29	-	-	-
30	-	-	-
31	Ramos BC 1	Ramos Barcode Pool 1	12/06/2024
32	Ramos BC 1	Ramos Barcode Pool 1	04/01/2023
33	Ramos BC 1	Ramos Barcode Pool 1 *	16/07/2024
34	Ramos BC 3	Ramos Barcode Pool 3	12/06/2024
35	Ramos BC 3	Ramos Barcode Pool 3	04/06/2024
36	Ramos BC 3	Ramos Barcode Pool 3	12/06/2024
37	Ramos BC 5	Ramos BC Pool 5	12/06/2024
38	Ramos BC 5	Ramos Barcode Pool 5	04/16/2024
39	Ramos BC 6	Ramos Barcode Pool 6	04/16/2024
40	-	-	-
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42	-	-	-
43	-	-	-
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45	-	-	-
46	-	-	-
47	-	-	-

Location	Cap ID	Description	Date
48	-	-	-
49	-	-	-
50	-	-	-
51	RBL1	RBL1 PDX	31/07/2023
52	RBL1	RBL1 PDX	31/07/2024
53	RBL1 PDX	RBL1 PDX	31/07/2024
54	BLLW	BLLW PDX Pool	31/07/2024
55	BLLW	BLLW PDX Pool	31/07/2024
56	N4	N4 PDX pool	07/11/2023
57	N4	N4 PDX pool	07/11/2023
58	N2 BC	N2 Barcoded pool	11/05/2023
59	N2 BC 5	N2 barcode pool 5	29/04/2024
60	A20	A20 Cell Pool	13/10/2024
61	A20	A20 Stock	13/10/2024
62	-	-	-
63	-	-	-
64	-	-	-
65	-	-	-
66	-	-	-
67	-	-	-
68	-	-	-
69	-	-	-
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86	-	-	-
87	-	-	-
88	-	-	-
89	-	-	-
90	-	-	-
91	NA	NA	NA
92	NA	NA	NA
93	NA	NA	NA
94	A20 ME	B-IP-724-1L	-
95	A20 ME	B-IP-723 NM	-
96	A20 ME	B-IP-723-2L	-
97	A20 ME	723-2R	-
98	A20 ME	723-1L	-
99	A20 ME	710 NM - A20 Mouse Experiment	-

Location	Cap ID	Description	Date
100	NA	NA	NA

January 2024

February 2024

March 2024

Monday 24-03-2024

MR - CD20 Flow EpiDrug Pretreatment

Protocol

1. 1x10e6 cells from each treatment group were split into 3 wells of a 96-well plate
2. Plate spun at 300xg 5 min and supernatant discarded
3. Cells were resuspnded in FACS Staining buffer

- 1ul CD20 (BD Cat# 562873) per 250uL
- 12 uL in 3000uL

4. Plate inbuated in the dark for 20min at 4C
5. Plate spun at 300xg 5 min and supernatant discarded
6. Samples were resuspended in 400uL PBS and transferred to FACS tubes

Results

- No clear alteration of CD20 expression due to Epigenetic drug pretreatment

Tuesday 25-03-2024

RTX EC50_250324 RBL2 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX on Baseline RBL2 in the presence of 25% NHS
- Seeded 1 plates with the same RBL2 Baseline population
- Used Rixathon (Catalogue#:)

Plate seeding protocol:

1. Diluted cell suspension to seed 10000 cells/well in 50μL amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1	RBL2	5.65×10^5	7.00×10^5	3.5	1.61×10^5	1238.9	2.2611

2. Made RTX dilutions and added to respective wells in 25μL
   - [RTX stock] = 10.3 mg/mL
   - Drug volumes are added in triplicate
   - Drug volumes are being added consititute 1/4 of well volume:

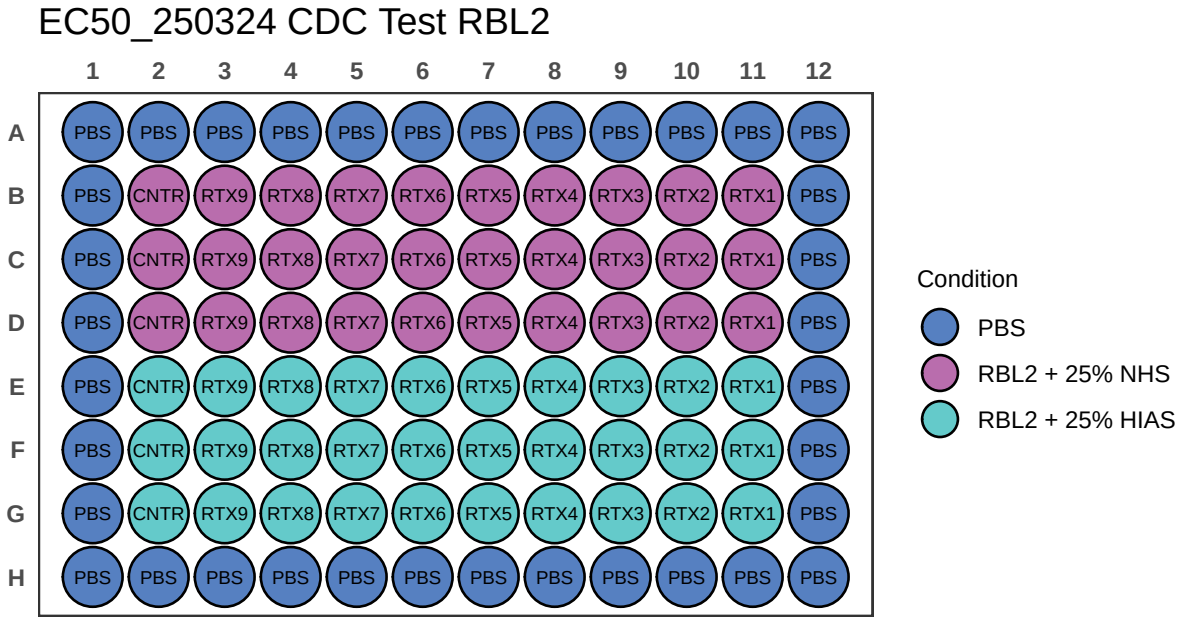
- [RTX working] needs to be 4x [RTX well]
  - 6 wells per condition, 25μL per well ~ minimum of 150μL per condition needed (recommend 200μL)

Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µg/mL)
RTX 1	20.0	Stock	4.6500000000000004	595.4	79.8
RTX 2	10.0	RTX 1	300	300.0	39.9
RTX 3	5.0	RTX 2	300	300.0	20.0
RTX 4	2.5	RTX 3	300	300.0	10.0
RTX 5	1.2	RTX 4	300	300.0	5.0
RTX 6	0.6	RTX 5	300	300.0	2.5
RTX 7	0.3	RTX 6	300	300.0	1.2
RTX 8	0.2	RTX 7	300	300.0	0.6
RTX 9	0.1	RTX 8	300	600.0	0.3
CNTR	0.0	-	-	1000.0	0.0

3. Added HIAS/NHS to indicated wells

- 25μL/well
- Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C



RBL2 RTX DP - Dose 1

- Began RTX CDC In Vitro dosing
- Seeded RBL2 into 2x 6 well plates

Dosing Protocol

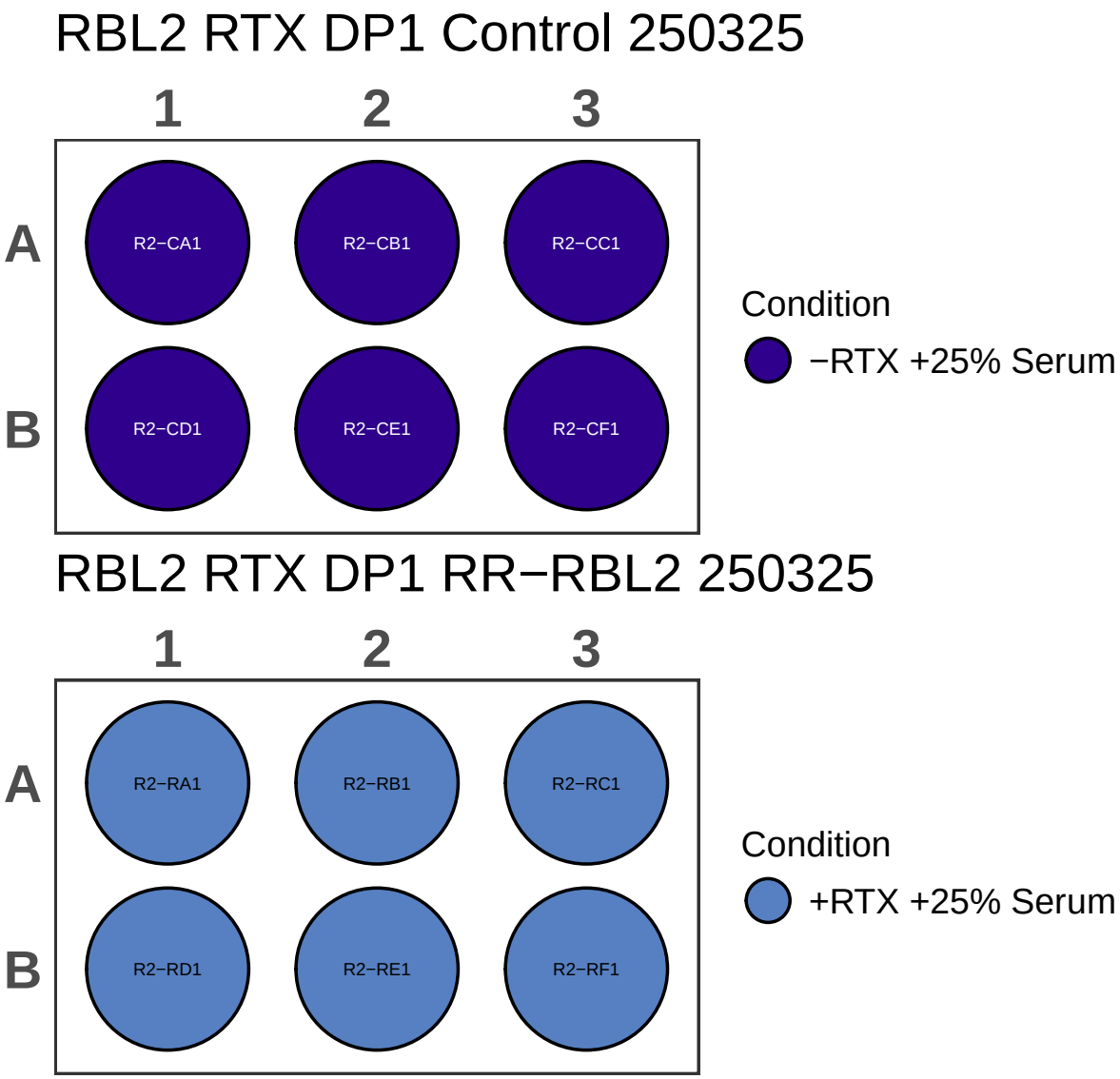
1. Count CS and dilute to 2x10^5 cells in 1 mL

- If cell count is below either re-culture or add required CS amount, spin down, and resuspend in 1mL

2. Add 1mL of cell suspension containing 2x10^5 cells to respective wells of 6-well plate

3. Made RTX dilutions and added to respective Rx wells in 500μL
   - [RTX stock] = 10.3 mg/mL
   - Drug volumes are being added consititute 1/4 of well volume:
     - [RTX working] needs to be 4x [RTX well]
   - 6 wells per RTX dosing, 500μL per well ~ minimum of 3000μL per condition needed (recommend 3500μL)
   - 1.4 uL RTX stock in 3.5mL media
   - 500μL media added to Cx wells
4. Added NHS to all wells
   - 500μL/well
   - Final well volume = 25% Serum (NHS)
4. Plates incubated for 24 hrs at 37C

Plate Layout



RBL2 RTX DP - Sample Collection Baseline

Collection Protocol

1. 1x10^6 cells added to eppendorf tubes
2. Spun down @ max speed @ 4C
3. Supernatant removed
4. Pellets flash frozen
5. Pellets saved at -80C

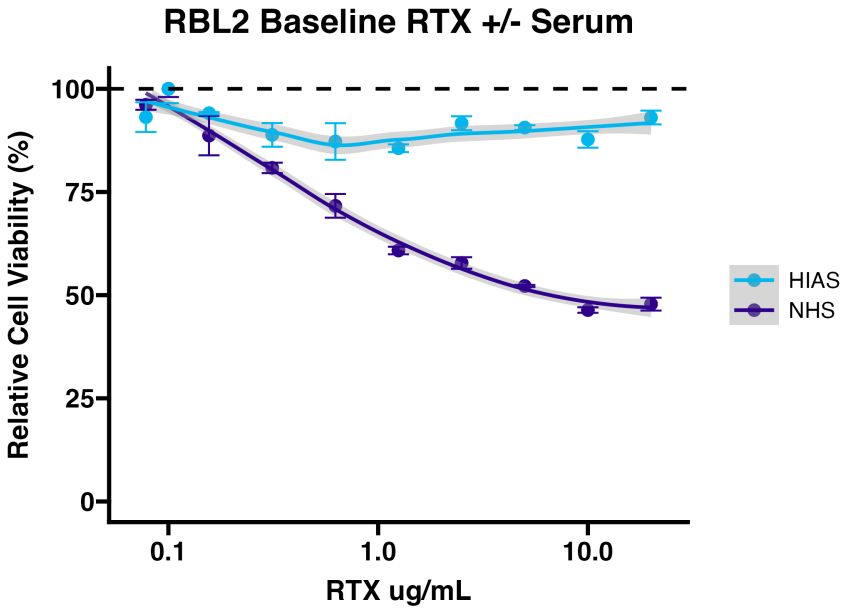
Thursday 27-03-2024

EC50_250325 Collection - RBL2 RTX CDC Baseline

- Collected plates seeded on 25-03-2025
- EC Plate collection protocol:
  1. Added 20μL Cell Titre Blue (CTB) to each conditioned well
  – 20μL CTB/100μL of conditioned well recommended by manufacturer
  2. Incubated for 2hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol

Results:

- HIAS impact on RBL2 CDC similar to RAMOS experiments
- NHS impact on RTX CDC is consistent with previous experiments
  – Cell viability was only reduced to 50%
  – Will repeat with higher doses of RTX (100ug/ml) and more careful cell counting



RBL2 RTX DP - Dose 1 Collection

Collection Protocol

1. Well volume transferred to 15ml Eppendorf
2. Eppendorfs supn down at 200 rcf for 6 min
3. Supernatant discarded and resuspended in 2ml

4. Collected cells added to individual wells of 6-well plates

Friday 28-03-2024

RAMOS-RTXDP SplitSeq: Qubit

- Needed to resumbit library due to low concentration

SS Qubit Results

Sample ID	DNA ng/uL	DNA nM
1	20.4	30.9
2	20.8	31.5
3	6.9	10.4
4	24.4	37.0
5	21.0	31.8
6	12.4	18.8
7	19.4	29.4
8	12.3	18.6

- CRUK-CI Genomics core tends to ask for 30-40uL of 10-20nM Library Pool

Monday 31-03-2024

RAMOS-RTXDP SplitSeq: Library Pooling and Submission

- Library Pooled for submission
- Submission ID: SLX-24264

Library Concentration (ng/µl)	Library Concentration (nM)	Library Volume (µl)	10 mM Tris-HCl, pH 8.5 (µl)	Pooling Volume (µl)
Library 1	30.9	10.3	2.2	4.9
Library 2	31.5	9.9	2.6	5.0
Library 3	10.6	0.0	0.0	11.8
Library 4	37.0	8.5	4.0	5.0
Library 5	31.8	9.8	2.7	5.0
Library 6	18.8	0.0	0.0	6.6
Library 7	29.4	10.6	1.9	5.0
Library 8	18.6	0.0	0.0	6.7

Total Pool Volume	Total Pool Concentration (ng/uL)
50	12.5

RTX EC50_250331 RBL2 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX on Baseline RBL2 in the presence of 25% NHS
- Seeded 1 plates with the RBL2 Baseline population
- Used Rixathon (Catalogue#:)

Plate seeding protocol:

1. Diluted cell suspension to seed 10000 cells/well in 50μL amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1	RBL2	1.26×10^6	7.00×10^5	3.5	3.60×10^5	555.5	2.9445

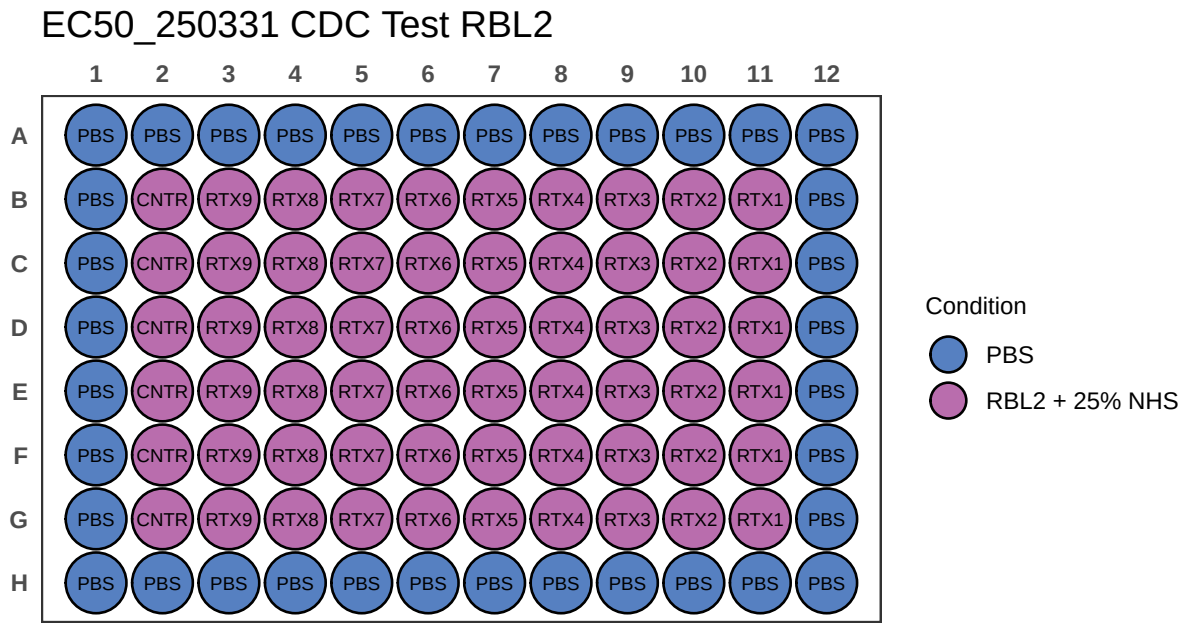
2. Made RTX dilutions and added to respective wells in 25μL
   - [RTX stock] = 10.3 mg/mL
   - Drug volumes are added in triplicate
   - Drug volumes are being added consititute 1/4 of well volume:
     - [RTX working] needs to be 4x [RTX well]
       - 6 wells per condition, 25μL per well ~ minimum of 150μL per condition needed (recommend 200μL)

Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µg/mL)
RTX 1	100.0	Stock	23.3	576.6	400.1
RTX 2	50.0	RTX 1	299.95	300.0	200.0
RTX 3	25.0	RTX 2	299.95	300.0	100.0
RTX 4	12.5	RTX 3	299.95	300.0	50.0
RTX 5	6.3	RTX 4	299.95	300.0	25.0
RTX 6	3.1	RTX 5	299.95	300.0	12.5
RTX 7	1.6	RTX 6	299.95	300.0	6.3
RTX 8	0.8	RTX 7	299.95	300.0	3.1
RTX 9	0.4	RTX 8	299.95	599.9	1.6
CNTR	0.0	-	-	1000.0	0.0

3. Added HIAS/NHS to indicated wells

- 25μL/well
- Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C



April 2025

Tuesday 01-04-2024

RTX EC50_250401 RBL2 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX on Baseline RBL2 in the presence of 25% NHS
- Seeded 1 plates with the RBL2 Baseline population
- Used Rixathon (Catalogue#:)

Plate seeding protocol:

1. Diluted cell suspension to seed 10000 cells/well in 50μL amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1	RBL2	9.70×10^5	7.00×10^5	3.5	2.77×10^5	721.6	2.7784

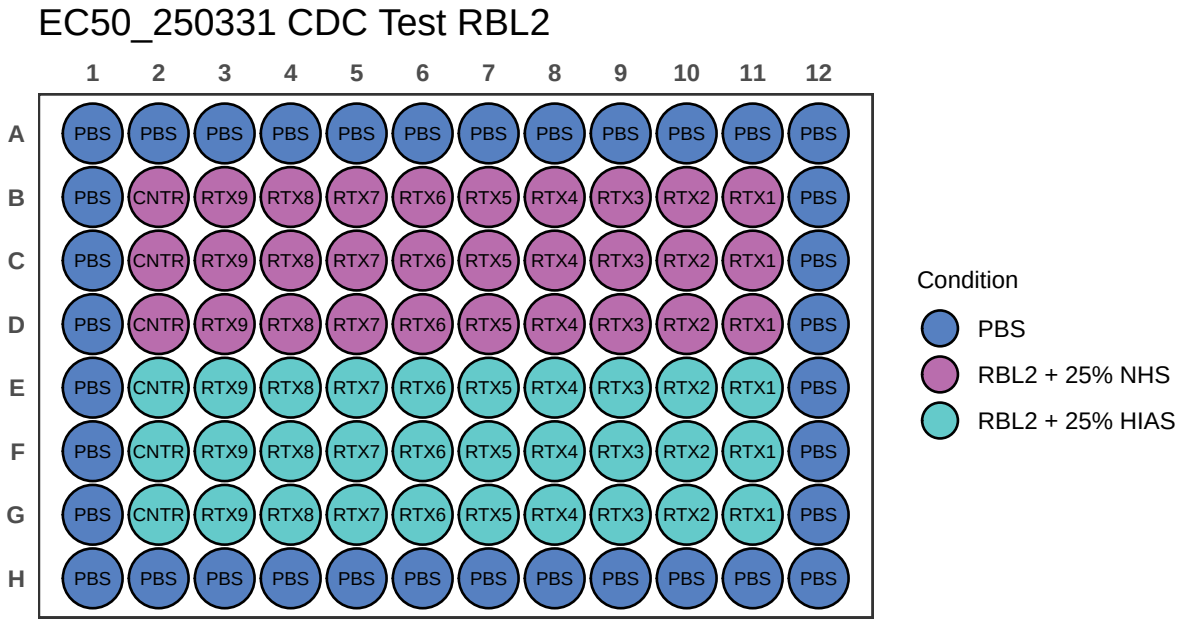
2. Made RTX dilutions and added to respective wells in 25μL
   - [RTX stock] = 10.3 mg/mL
   - Drug volumes are added in triplicate
   - Drug volumes are being added consititute 1/4 of well volume:
     - [RTX working] needs to be 4x [RTX well]
       - 6 wells per condition, 25μL per well ~ minimum of 150μL per condition needed (recommend 200μL)

Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µg/mL)
RTX 1	100.0	Stock	23.3	576.6	400.1
RTX 2	50.0	RTX 1	299.95	300.0	200.0
RTX 3	25.0	RTX 2	299.95	300.0	100.0
RTX 4	12.5	RTX 3	299.95	300.0	50.0
RTX 5	6.3	RTX 4	299.95	300.0	25.0
RTX 6	3.1	RTX 5	299.95	300.0	12.5
RTX 7	1.6	RTX 6	299.95	300.0	6.3
RTX 8	0.8	RTX 7	299.95	300.0	3.1
RTX 9	0.4	RTX 8	299.95	599.9	1.6
CNTR	0.0	-	-	1000.0	0.0

3. Added HIAS/NHS to indicated wells

- 25μL/well
- Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C



RBL2 RTX DP - Dose 1

- Restarted RTX CDC In Vitro dosing
- Concerns with initial seeding/dosing
  - RBL2 appear to be more resistant to RTX dosing
  - Plan is to start with 25ug/mL RTX
  - 25 -> 50 -> 100 -> 200
- Seeded RBL2 into 2x 6 well plates

Dosing Protocol

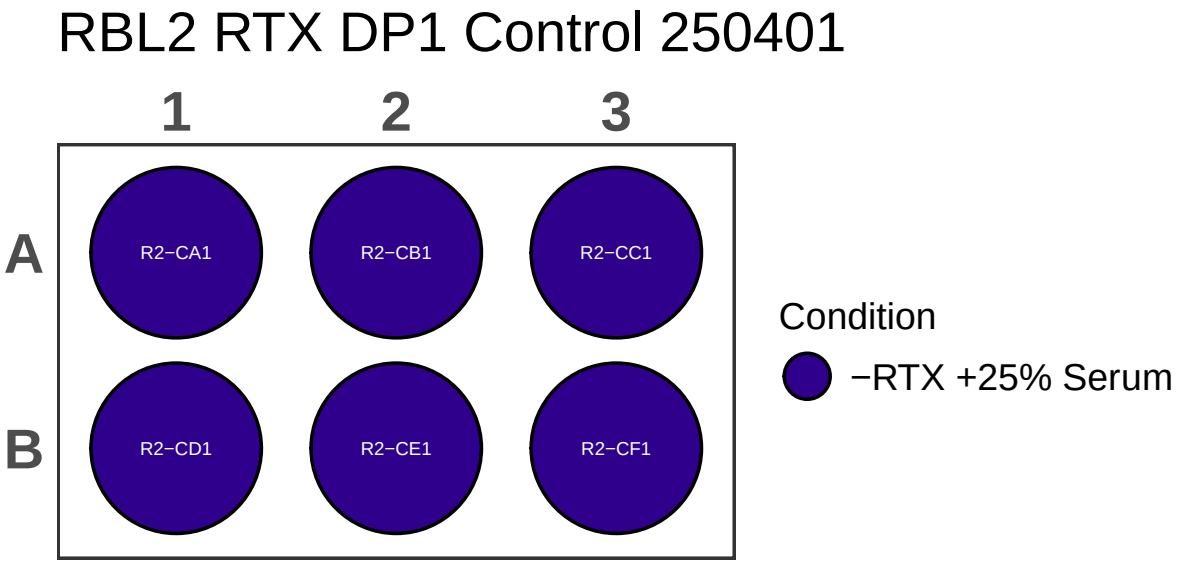
1. Count CS and dilute to 5x10^5 cells in 1 mL

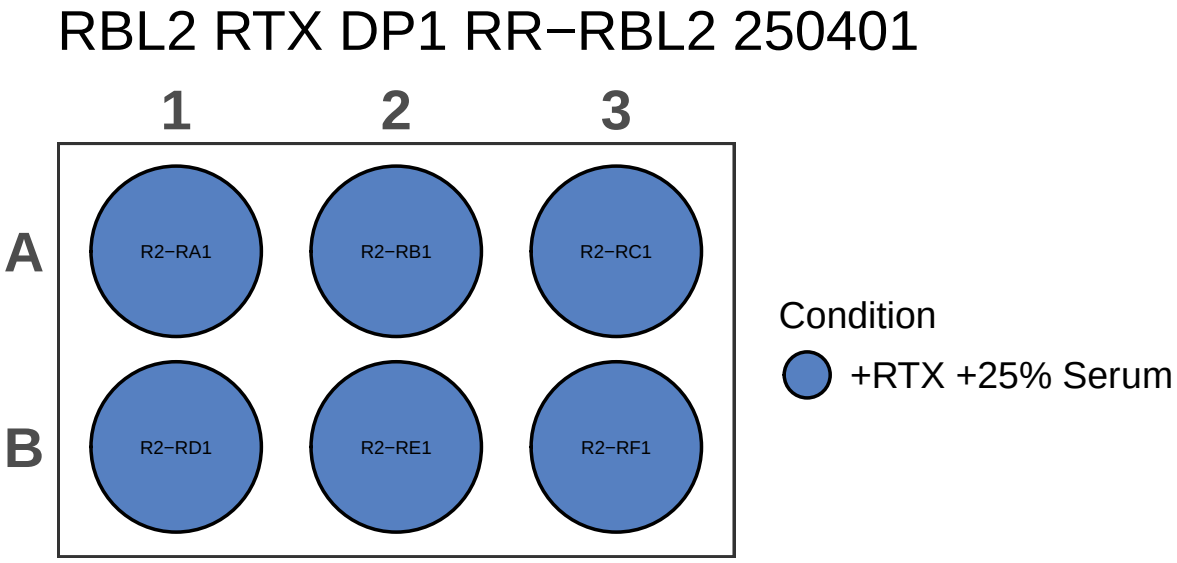
- If cell count is below either re-culture or add required CS amount, spin down, and resuspend in 1mL

2. Add 1mL of cell suspension containing 5x10^5 cells to respective wells of 6-well plate

3. Made RTX dilutions and added to respective Rx wells in 500μL
   - [RTX stock] = 10.3 mg/mL
   - Drug volumes are being added consititute 1/4 of well volume:
     - [RTX working] needs to be 4x [RTX well]
   - 6 wells per RTX dosing, 500μL per well ~ minimum of 3000μL per condition needed (recommend 3500μL)
   - 25ug/mL [RTX]
     - 34μL RTX stock in 3.5mL media
     - 100ug/mL WS
   - 500μL media added to Cx wells
4. Added NHS to all wells
   - 500μL/well
   - Final well volume = 25% Serum (NHS)
4. Plates incubated for 24 hrs at 37C

Plate Layout





Wednesday 02-04-2024

Media Prep

Advanced RPMI for PDX

- 20% FBS #### Recipe

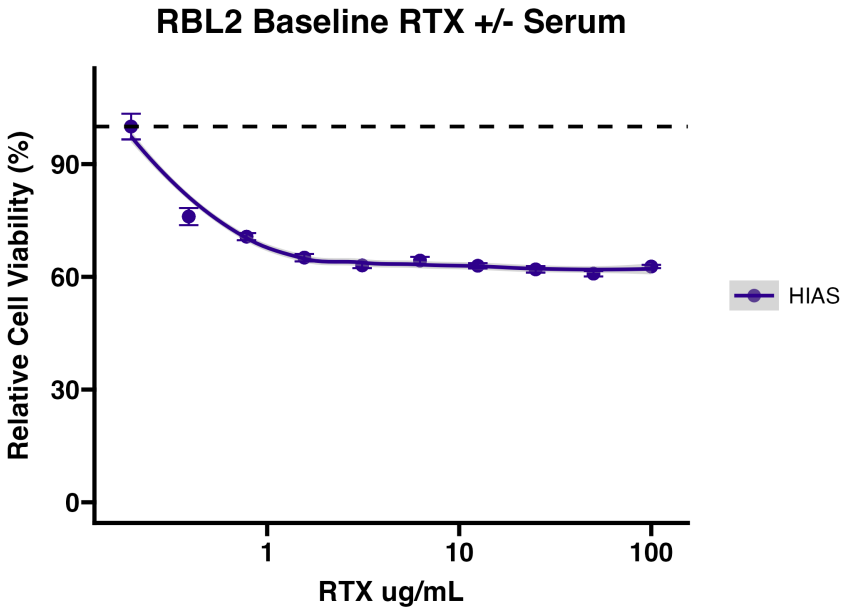
Solution	ID code	Volume	% Total volume
Advanced RMPI	—-	500 mL	78%
Glutamax	—-	6.5 mL	20%
Pen-Strep	—-	6.5 mL	1%
FBS	—-	128 mL	20%

EC50_250331 Collection - RBL2 RTX CDC Baseline

- Collected plates seeded on 25-03-2025
- EC Plate collection protocol:
  1. Added 20μL Cell Titre Blue (CTB) to each conditioned well
  - 20μL CTB/100μL of conditioned well recommended by manufacturer
  2. Incubated for 2hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol

Results:

- Forgot to seed HIAS
- NHS impact on RTX CDC is consistent with previous experiments
  - Cell viability still wasnt reduced below 50%
  - Will repeat with higher doses of RTX (1000ug/ml) and increased cell/well amount (4x10^5)



RTX EC50_250402 RBL2 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX on Baseline RBL2 in the presence of 25% NHS
- Seeded 1 plates with the RBL2 Baseline population
- Used Rixathon (Catalogue#:)

Plate seeding protocol:

1. Diluted cell suspension to seed 40000 cells/well in 50μL amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1	RBL2	9.70×10^5	7.00×10^5	3.5	2.77×10^5	721.6	2.7784

2. Made RTX dilutions and added to respective wells in 25μL
   - [RTX stock] = 10.3 mg/mL
   - Drug volumes are added in triplicate
   - Drug volumes are being added consititute 1/4 of well volume:
     - [RTX working] needs to be 4x [RTX well]
       - 6 wells per condition, 25μL per well ~ minimum of 150μL per condition needed (recommend 200μL)

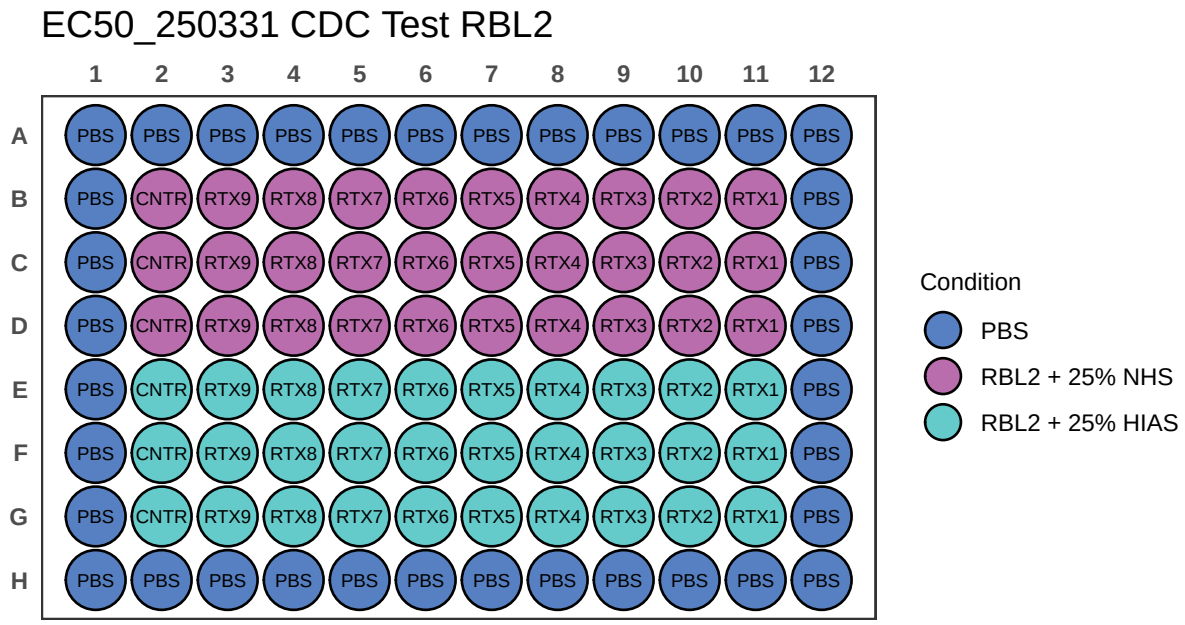
Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µg/mL)
RTX 1	100.0	Stock	23.3	576.6	400.1
RTX 2	50.0	RTX 1	299.95	300.0	200.0
RTX 3	25.0	RTX 2	299.95	300.0	100.0
RTX 4	12.5	RTX 3	299.95	300.0	50.0
RTX 5	6.3	RTX 4	299.95	300.0	25.0

Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µg/mL)
RTX 6	3.1	RTX 5	299.95	300.0	12.5
RTX 7	1.6	RTX 6	299.95	300.0	6.3
RTX 8	0.8	RTX 7	299.95	300.0	3.1
RTX 9	0.4	RTX 8	299.95	599.9	1.6
CNTR	0.0	-	-	1000.0	0.0

3. Added HIAS/NHS to indicated wells

- 25μL/well
- Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C



Thursday 03-04-2024

RBL2 RTX DP - Dose 1 Collection

Collection Protocol: RTX Treated

1. Well volume transferred to 15ml Eppendorf
2. Eppendorfs supn down at 200 rcf for 6 min
3. Supernatant discarded and resuspended in 1ml
4. Collected RTX-treated cells added to individual wells of 24-well plates

- Control wells expanded to T25s in 4ml media

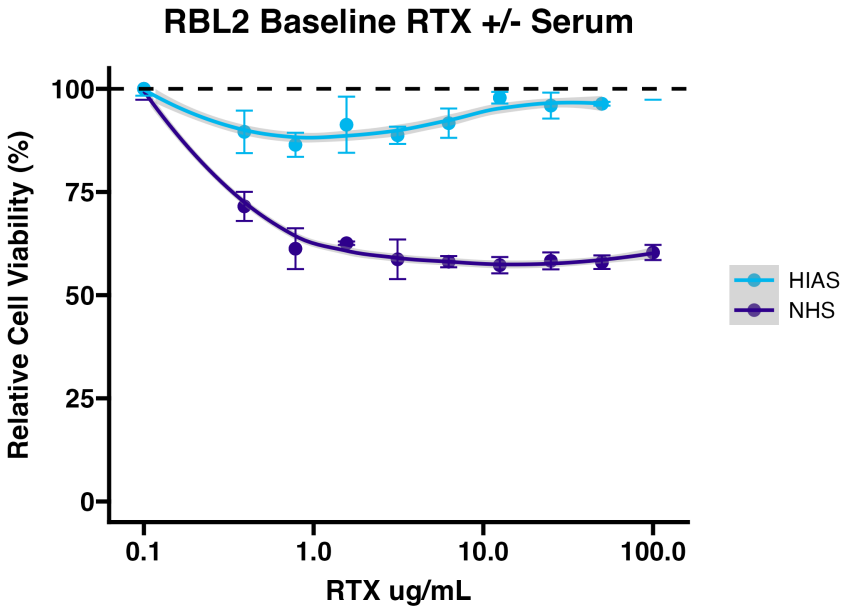
EC50_250401 Collection - RBL2 RTX CDC Baseline

- Collected plates seeded on 25-03-2025
- EC Plate collection protocol:
  1. Added 20μL Cell Titre Blue (CTB) to each conditioned well
  – 20μL CTB/100μL of conditioned well recommended by manufacturer
  2. Incubated for 2hr at 37C

3. Read on plate reader according to Cell Titre Blue Protocol

Results:

- HIAS Results as expected
  - Need to seed one more as reference
- NHS impact on RTX CDC is consistent with previous experiments
  - Cell viability still wasnt reduced below 50%
  - Will repeat with higher doses of RTX (1000ug/ml) and increased cell/well amount ($4x10^5$)



Friday 04-04-2024

RBL2 RTX DP - Dose 1 Culture

RBL2 RTX-Treated

- Expanded to T25
- Transferred 1mL of CS and added 3mL of fresh media
- Cells looked relatively healthy, good growth from yesterday

RBL2 Control

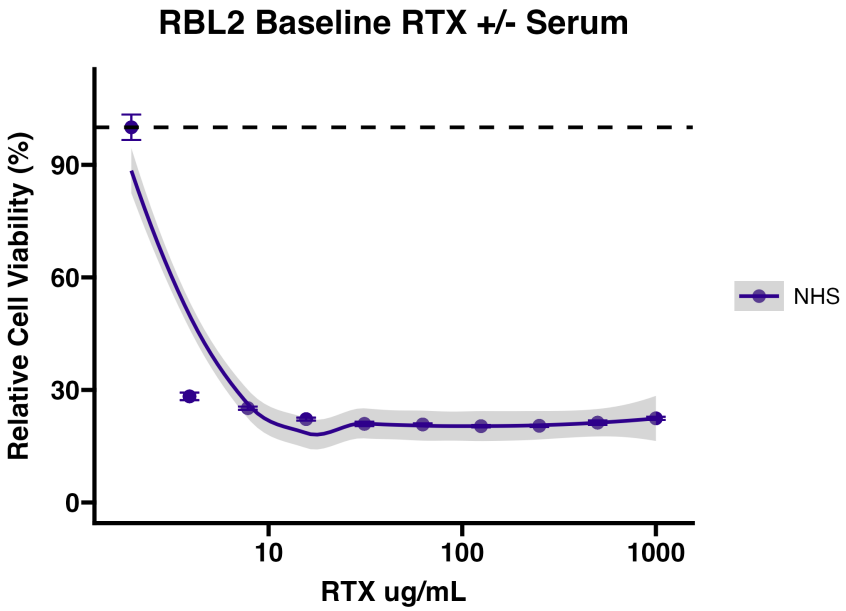
- Spun down and resuspended in 4 ml of media
- Look fine

EC50_250402 Collection - RBL2 RTX CDC Baseline

- Collected plates seeded on 02-04-2025
- EC Plate collection protocol:
  1. Added 20μL Cell Titre Blue (CTB) to each conditioned well
  - 20μL CTB/100μL of conditioned well recommended by manufacturer
  2. Incubated for 2hr at 37C
  3. Read on plate reader according to Cell Titre Blue Protocol

Results:

- NHS impact on RTX CDC is wildly different
  - Down to 30% viability at ~4ug/mL
  - Seems like the issue was cell seeding
  - Will repeat 07-04-2025 with 20ug/mL but 4x10^5 cells



Friday 04-04-2024

RBL2 RTX DP - Dose 1 Culture

RBL2 RTX-Treated

- Cells look rough
- Spin down and resuspend in 12 well plates
- Expanded too quickly

RBL2 Control

- Look good
- Expand to T75

RTX EC50_250407 RBL2 - Seeding

- Seeded an EC50 experiment comparing the effects of RTX on Baseline RBL2 in the presence of 25% NHS or 25% HIAS
  - Looking to establish optimal EC50 range
- Seeded 1 plates with the RBL2 Baseline population based on previous results:
  - 40k cells per well
  - Starting at 100ug/mL dose
- Used Rixathon (Catalogue#:)

Plate seeding protocol:

1. Diluted cell suspension to seed 40000 cells/well in 50μL amounts

Plate	Cell Line	Cell Count	Required Cell total	Required Volume total	CS cells/mL	Stock Volume (uL)	Media Volume (mL)
Plate 1	RBL2	1.63×10^6	2.80×10^6	4	4.08×10^5	1717.7	2.2823

2. Made RTX dilutions and added to respective wells in 25μL
   - [RTX stock] = 10.3 mg/mL
   - Drug volumes are added in triplicate
   - Drug volumes are being added consititute 1/4 of well volume:
     - [RTX working] needs to be 4x [RTX well]
       - 6 wells per condition, 25μL per well ~ minimum of 150μL per condition needed (recommend 200μL)

Dilution ID	Well [RTX] (µg/mL)	RTX Source	Source Volume (µL)	Media Volume (µL)	Working Stock [RTX] (µg/mL)
RTX 1	100.0	Stock	19.417475728155338	480.6	400.0
RTX 2	50.0	RTX 1	250	250.0	200.0
RTX 3	25.0	RTX 2	250	250.0	100.0
RTX 4	12.5	RTX 3	250	250.0	50.0
RTX 5	6.2	RTX 4	250	250.0	25.0
RTX 6	3.1	RTX 5	250	250.0	12.5
RTX 7	1.6	RTX 6	250	250.0	6.2
RTX 8	0.8	RTX 7	250	250.0	3.1
RTX 9	0.4	RTX 8	250	500.0	1.6
CNTR	0.0	-	-	1000.0	0.0

3. Added HIAS/NHS to indicated wells

- 25μL/well
- Final well volume = 25% Serum (HIAS/NHS)

4. Plate is incubated for 48 hrs at 37C