

## *CELL310: RETМ MEDIA RECIPE*

Shorthand Name: **RETМ**

Media Full Name: **Renaissance Tumor Media**

Updated: **9/14/2023 by Hannah Golding**

Reagent	Manufacturer	Cat. No	Type Annotation	Volume	Final Concentration
Hams F-12 nutrient mix	Thermo Fisher	11765054	Basal Medium	489mL	50% v/v
Medium 199, Earle's Salts	Thermo Fisher	11150059	Basal Medium	489mL	50% v/v
<i>*RETМ Supplement Mix</i>	<i>*In house</i>	-	Supplement Mix	10mL	-
Pen/Strep/Glut (100X)	Invitrogen	10378-016	Antibiotics	10mL	1X
Fungizone (250 µg/mL)	Invitrogen	15290018	Antibiotics	2mL	0.5 ug/mL
Gentamicin (50 mg/mL)	Life Technologies	15750-060	Antibiotics	200uL	10 ug/mL
3,3',5-Triiodo-L-thyronine (10 µM)	Sigma-Aldrich	T6397	Reagent	2mL	0.2 pg/mL
apo-Transferrin human	Sigma-Aldrich	T5391	Reagent	1mL	10 ug/mL
Beta-estradiol (20 µM)	Sigma-Aldrich	E8875-250MG	Reagent	12.5µL	0.5 ng/mL
Bovine serum albumin (BSA) (2 mg/mL)	Thermo Fisher	23209	Serum	1.65mL	3.3 ug/mL
Cholera toxin (1 mg/mL)	Sigma-Aldrich	C-8052-2MG	Reagent	100uL	100 ng/mL
EGF (10 µg/mL)	Gibco	PHG0314	Reagent	50µL	0.5 ng/mL
FBS	Sigma-Aldrich	F4135	Serum	50mL	5%
HEPES (10 mM)	Sigma-Aldrich	H0887	Reagent	10mL	0.1 mM
Insulin (5 mg/mL)	Thermo Fisher	12585014-5mL	Reagent	2mL	10 ug/mL
L-Glutathione reduced	Sigma-Aldrich	G4251-1G	Reagent	12µL	0.012 ug/mL
O-Phosphorylethanolamine	Sigma-Aldrich	P0503	Reagent	500µL	5 ug/mL

Note: Antibiotics must be added for lines in the nursery stage. Established cultures do not need antibiotics.

1. To the basal medium, aseptically add the Pen/Strep/Glut, Fungizone, and Gentamicin.
2. Media should be made in fresh batches every 90 days. Any media older than 3 months must be discarded.

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**RETM Supplement Mix**

#	Reagent	Manufacturer	Cat. No	Final Concentration of Supplement Mix	Solvent
1	<b>4-Aminobenzoic acid</b>	Sigma-Aldrich	A9878-25G	0.012 ug/mL	water
2	<b>Calciferol (vitamin D2)</b>	Sigma-Aldrich	V-024-1mL	0.025 ug/mL	EtOH or DMSO
3	<b>Cholesterol</b>	Sigma-Aldrich	C3045	0.05 ug/mL	EtOH
4	<b>Choline chloride</b>	Sigma-Aldrich	C7527	3.5 ug/mL	water
5	<b>Folic acid</b>	Thermo Fisher	216630500	0.33 ug/mL	NaOH
6	<b>Glutamine</b>	Thermo Fisher	A2916801	2 mM	-
7	<b>Hydrocortisone</b>	Sigma-Aldrich	H0888	0.5 ng/mL	-
8	<b>Hypoxanthine</b>	Thermo Fisher	A11481.06	1.75 ug/mL	1:3 DMSO:PBS
9	<b>i-Inositol</b>	Selleck Chemicals	S4530	4.5 ug/mL	water
10	<b>L-Ascorbic acid</b>	Sigma-Aldrich	A4544-25G	0.012 ug/mL	water
11	<b>Linoleic acid</b>	Sigma-Aldrich	L1012-100MG	5 ug/mL	EtOH
12	<b>Lipoic acid</b>	Sigma-Aldrich	T1395-1G	0.05 ug/mL	1:8 EtOH:PBS
13	<b>Retinoic acid</b>	Sigma-Aldrich	R2625-50MG	0.025 ug/mL	EtOH or DMSO
14	<b>Ribose</b>	Sigma-Aldrich	R9629-25G	0.125 ug/mL	water
15	<b>Selenious acid</b>	Thermo Fisher	12574.06	8 ng/mL	water
16	<b>Thiamine HCL</b>	Sigma-Aldrich	V-014-1mL	0.08 ug/mL	water
17	<b>Tocopherol phosphate</b>	Sigma-Aldrich	T2020-250MG	0.003 ug/mL	water
18	<b>Uracil</b>	Sigma-Aldrich	U1128-25G	0.075 ug/mL	NaOH
19	<b>Vitamin B12</b>	Sigma-Aldrich	V6629-100MG	0.35 ug/mL	water
20	<b>Xanthine</b>	Sigma-Aldrich	X4002-1G	0.085 ug/mL	1M NaOH

Note: RETM supplement mix is to be prepared beforehand and kept in 10mL aliquots to be used to make 1L of media (stored at -20C)

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**Stock Preparation and Storage for Media Reagents**

<b>Reagent</b>	<b>Stock Preparation</b>	<b>Stock Concentration</b>	<b>Stock Storage</b>
<b>Fungizone (250 µg/mL)</b>	Aliquot 1 mL into Eppendorf tubes	250 µg/mL	-20C
<b>Gentamicin (50 mg/mL)</b>	-	50 mg/mL	Room Temp
<b>Pen/Strep/Glut (100X)</b>	Aliquot 10 mL into 15 mL conical tubes	100x	-20C
<b>3,3',5-Triiodo-L-thyronine</b>	Dilute 1.37 mg in 2 mL 1M NaOH. Dilute 1:100 by sterile medium	10 µM	-20C
<b>apo-Transferrin human</b>	Resuspend 10 mg in 1 mL dH <sub>2</sub> O	10 mg/mL	-20C
<b>Beta-estradiol</b>	Resuspend 1g in 185µL in PBS	20 µM	-20C
<b>Cholera toxin</b>	Resuspend 2mg in 2mL dH <sub>2</sub> O	1 mg/mL	4C
<b>EGF</b>	Resuspend lyophilized EGF in 1 mL PBS	10 µg/mL	4C
<b>Insulin</b>	Prepare 1mL aliquots	5 mg/mL	-20C
<b>L-Glutathione reduced</b>	15.625mg in 15.625mL in EtOH; make fresh every time	0.012 ug/mL	Make fresh
<b>O-Phosphorylethanolamine</b>	Resuspend 14.1 g in 1 mL dH <sub>2</sub> O. Dilute 1:10 in dH <sub>2</sub> O	100 mM	-20C