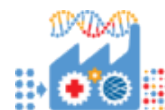


## AR5



## LIMS NAMING: AR5 Media

## FULL MEDIA NAMING: ACL4 Media with 5% FBS

Reagent	Manufacturer	Catalogue #	Type Annotation	500mL	1L	Final Concentration
RPMI with L-Glutamine	VWR International	45000-398	Basal Medium	437 mL	874 mL	93%
FBS	Sigma Aldrich	F2442	Serum	25 mL	50 mL	5%
BSA (40 mg/mL)	Seracare Life Sciences Inc.	AP-4510-80	Serum	25 mL	50 mL	2 mg/mL
HEPES (1 M)	Sigma Aldrich	HO887	Supplement	5 mL	10 mL	1%
Pen/Strep (100 x)*	Invitrogen	15140-163	Antibiotics	5 mL	10 mL	1%
Sodium Pyruvate (100 mM)	Sigma Aldrich	S8636	Supplement	2.5 mL	5 mL	500 µM
Insulin (5 mg/mL)	Sigma Aldrich	I2643	Supplement	2 mL	4 mL	20 µg/mL
Fungizone (250 ug/mL)*	Invitrogen	15290018	Antibiotics	1 mL	2 mL	500 ng/mL
Transferrin (10 mg/mL)	Sigma Aldrich	T5391	Supplement	500 µL	1 mL	10 µg/mL
EGF (10 µg/mL)	Life Technologies	PHG0314	Supplement	500 µL	1 mL	10 ng/mL
Ethanolamine (10 mM)	Sigma Aldrich	411000	Supplement	500 µL	1 mL	10 µM
O-Phosphorylethanolamine (0.1 M)	Sigma Aldrich	P0503	Supplement	500 µL	1 mL	10 µM
3,3',5-Triiodo-L-thyronine (0.1 µM)	Sigma Aldrich	T6397	Supplement	500 µL	1 mL	0.1 nM
Sodium Selenite (30 µM)	Sigma Aldrich	S5261	Supplement	415 µL	830 µL	25 nM
Gentamicin (50 mg/mL)*	Life Technologies	15750-060	Antibiotics	100 µL	200 µL	20 µg/mL
Hydrocortisone (0.1 mM)	Sigma Aldrich	H0888	Supplement	250 µL	500 µL	50 nM

\* Antibiotics must be added for lines in nursery. Established cultures do not need antibiotics.

1. Filter RPMI, FBS, BSA, HEPES, Pen/Strep, and sodium pyruvate through 0.22 µm cellulose nitrate vacuum filter.
2. Aseptically add insulin, Fungizone, transferrin, EGF, ethanolamine, O-phosphorylethanolamine, 3,3',5-triiodo-L-thyronine, sodium selenite, Gentamicin, and hydrocortisone to the filtered solution.
3. Media should be made in fresh batches every two weeks. Any media over a month old should be discarded.



## Stock Preparation and Storage for Media Reagents

Reagent	Stock preparation	Stock concentration	Stock storage
RPMI with L-Glutamine	-	-	4° C
FBS	Aliquot 50 mL into 50 mL conical tubes.	-	-20° C
BSA	Resuspend 2 g in 50 mL of dH <sub>2</sub> O	40 mg/mL	-20° C
HEPES Buffer*	-	1 M	4° C
Pen/Strep	Aliquot 10 mL into 15 mL conical tubes.	100 X	-20° C
Sodium Pyruvate*	-	100 mM	4° C
Insulin	50 mg in mix of 100 µL glacial acetic acid and 10 mL dH <sub>2</sub> O	5 mg/mL	-20° C
Fungizone	Aliquot 1 mL into 1.5 mL Eppendorf tubes.	250 µg/mL	-20° C
Transferrin*	Resuspend 10 mg in 1 mL dH <sub>2</sub> O.	10 mg/mL	-20° C
EGF	-	10 µg/mL	-20° C
Ethanolamine*	6.02 µL of 16.6 M solution in 10 mL dH <sub>2</sub> O (add fresh) Can be diluted and stored at -20°C	10 mM	RT
O-Phosphorylethanolamine*	14.1 mg in 10mls RPMI basal media. Mix until dissolved	10 mM	-20° C
3,3',5-triiodo-L-thyronine*	1.37 mg in 2 mL 1 M NaOH, then 1:100 dilution by sterile medium, then 1:100 dilution by sterile medium	0.1 µM	-20° C
Sodium Selenite*	100 mg in 193 mL dH <sub>2</sub> O, then 1:10 dilution in dH <sub>2</sub> O	30 µM	-20° C
Gentamicin	-	50 mg/mL	RT
Hydrocortisone*	1mg in 50 mL ethanol, then add 26.5ml of RPMI basal media and mix well	0.1 mM	-20° C

\* External orders

SOP #	SOP Name	Date	Version	SOP Owner
CELL301	AR5	2020/09/10	1	Rebecca Deasy