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SJB Artificial Seawater Medium Protocol

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We use this protocol and it's working

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Abstract

SJB Artificial Seawater Medium Protocol

SJB Artificial Seawater Medium is an amended version of JW and MWH generations of Thrash Lab artificial seawater medium.

Materials

Sterilized Pyrex Bottle

0.2um PES filters

Base Salts

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
	Salinity			***1***		***2***		***3***		***4***		***4.25***		***4.5***		***5***	
	Base Salts	**Chemical Formula**	**Formula Weight**	**g/L**	**Final M**	**g/L**	**Final M**	**g/L**	**Final M**	**g/L**	**Final M**	**g/L**	**Final M**	**g/L**	**Final M**	**g/L**	**Final M**
	sodium chloride	NaCl	58.443	23.5	0.402101	15.7	0.268067	7.83333	0.134033667	3.91666667	0.067016833	3.012820513	0.05155141	2.008547009	0.034367607	1.004273504	0.017183803
	potassium chloride	KCl	74.55	0.746	0.010007	0.497	0.006671333	0.248666667	0.003335667	0.124333333	0.001667833	0.095641026	0.001282949	0.063760684	0.000855299	0.031880342	0.00042765
	sodium bicarbonate	NaHCO3	84.01	0.84	0.009999	0.84	0.006666	0.42	0.003333	0.21	0.0016665	0.161538462	0.001281923	0.107692308	0.000854615	0.053846154	0.000427308
	sodium sulfate	Na2SO4	142.04	4.27	0.030062	2.85	0.020041333	1.42333333	0.010020667	0.71166667	0.005010333	0.547435897	0.003854103	0.364957265	0.002569402	0.182478632	0.001284701
	sodium bromide	NaBr	102.89	0.83	0.008067	0.55	0.005378	0.276666667	0.002689	0.138333333	0.0013445	0.106410256	0.001034231	0.070940171	0.000689487	0.035470085	0.000344744
	boric acid	H3BO3	61.83	0.026	0.00421	0.017	0.00280667	0.008666667	0.00140333	0.004333333	7.01667E-05	0.003333333	5.39744E-05	0.002222222	3.59829E-05	0.001111111	1.79915E-05

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
	strontium chloride	SrCl	158.53	0.0142	0.00009	0.009467	0.00006	0.004733333	0.00003	0.002366667	0.000015	0.001820513	1.15385E-05	0.001213675	7.69231E-06	0.000606838	3.84615E-06
	sodium flouride	NaF	41.99	0.0023	0.000055	0.0015	3.66667E-05	0.000766667	1.83333E-05	0.000383333	9.16667E-06	0.000294872	7.05128E-06	0.000196581	4.70085E-06	9.82906E-05	2.35043E-06
	magnesium chloride heptahydrate	MgCl2 x 6H2O	203.3	10.6	0.05214	7.1	0.03476	3.533333333	0.01738	1.766666667	0.00869	1.358974359	0.006684615	0.905982906	0.004456641	0.452991453	0.002228205
	calcium chloride dihydrate	CaCl2 x 2H2O	147.01	1.52	0.010339	1.013	0.006892667	0.506666667	0.003446333	0.253333333	0.001723167	0.194871795	0.001325513	0.12991453	0.000883675	0.064957265	0.000441838

Table 1. Base Salts
The range of salinities are shown in order of highest (#1) to lowest (#5).

Trace Metal Mix (100,000x Stock)

	A	B	C	D	E
	Compound	**Chemical Formula**	**FW**	**g/100mL**	**Final M (nM)**
	manganese dichloride tetrahydrate	MnCl2 × 4H2O	197.91	0.018	9
	zinc sulfate monohydrate	ZnSO4 X H2O	179.47	0.002	1

	A	B	C	D	E
	cobalt(II) chloride	CoCl2	129.839	0.001	0.5
	sodium molybdate	Na2MoO4	205.92	0.001	0.3
	sodium selenite	Na2SeO3	172.94	0.002	1
	nickel(II) chloride	NiCl2	129.5994	0.001	1

Vitamin Mix (100,000x)

	A	B	C	D	E
	Compound	**Chemical Formula**	**FW**	**g/100mL**	**Final M (nM)**
	B1/thiamine	C12H17ClN4OS · HCl	337.27	1.69	500 nM
	B2/riboflavin	C17H20N4O6	376.36	0.0026	0.7 nM
	B3/niacin	C6H5NO2	123.12	0.985	800 nM
	B5/pantothenate	HOCH2C(CH3)2CH(OH)CONHCH2CH2CO2·1/2Ca	238.27	1.013	425 nM
	B6/pyridoxine	C8H11NO3 · HCl	205.64	1.028	500 nM
	B7/biotin	C10H16N2O3S	244.31	0.0098	4 nM
	B9/folic Acid	C19H19N7O6	441.4	0.0177	4 nM
	B12/cyanocobalamin	C63H88CoN14O14P	1355.37	0.0095	0.7 nM
	myo-inositol	C6H12O6	180.16	0.901	500 nM
	4-aminobenzoic Acid	C7H7NO2	137.14	0.0823	60 nM

Amino Acid Mix (5,000x)

MEM Amino Acids (50x) Solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M5550- 100mL

	A	B	C	D	E
	Compound	**Chemical Formula**	**FW**	**g/100mL**	**Final M**
	L-arginine x HCl	HN=C(NH2)NH(CH2)3CH(NH2)COOH · HCl	210.7	0.632	0.0000005999050783
	L-cystine x 2HCl	C6H12N2O4S2 · 2HCl	313.22	0.1564	0.00000009986590895
	L-histidine x HCl x H2O	C6H9N3O2 · HCl · H2O	209.65	0.21	0.0000002003338898
	L-isoleucine	C2H5CH(CH3)CH(NH2)CO2H	131.17	0.2625	0.0000004002439582
	L-leucine	CH3)2CHCH2CH(NH2)CO2H	131.17	0.262	0.0000003994815888
	L-lysine x HCl	H2N(CH2)4CH(NH2)CO2H·HCl	182.69	0.3625	0.0000003968471181
	L-methionine	CH3SCH2CH2CH(NH2)CO2H	149.21	0.0755	0.0000001011996515
	L-phenylalanine	C6H5CH2CH(NH2)CO2H	165.19	0.165	0.0000001997699619
	L-threonine	CH3CH(OH)CH(NH2)CO2H	119.12	0.238	0.000000399597045
	L-tryptophan	C11H12N2O2	204.23	0.051	0.00000004994369094
	L-tyrosine	4-(HO)C6H4CH2CH(NH2)CO2H	181.19	0.18	0.0000001986864617
	L-valine	(CH3)2CHCH(NH2)CO2H	117.15	0.234	0.0000003994878361

Miscellaneous Mix (20,000x)

	A	B	C	D	E	F
	Compound	**Chemical Formula**	**FW**	**g/100mL**	**mL/100mL**	**Final M**
	L-glutamine	C5H10N2O2	146.14	0.14614		0.0000005
	dextrose	C6H12O6	180.16	0.18016		0.0000005

	A	B	C	D	E	F
	D-ribose	C5H10O5	150.13	0.15013		0.0000005
	sodium pyruvate	C3H3NaO3	110.04	0.11004		0.0000005
	sodium citrate	C6H5Na3O7	294.1	0.2941		0.0000005
	oxaloacetic acid	C4H4O5	132.07	0.13207		0.0000005
	sodium acetate	C2H3NaO2	82.03	0.08203		0.0000005
	sodium succinate dibasic hexahydrate	NaOOCCH2CH2COONa · 6H2O	270.14	0.27014		0.0000005
	a-ketoglutaric acid	C5H6O5	168.08	0.16808		0.0000005
	urea	CH4N2O	60.6	0.606		0.000005
	gylcerol	C3H8O3	92.09	0.09209		0.0000005
	glycine betaine	C5H11NO2	153.61	0.15361		0.0000005
	choline	(CH3)3N(Cl)CH2CH2OH	139.62	0.13962		0.0000005
	sodium thiosulfate	Na2S2O3	158.11	0.15811		0.0000005
	potassium cyanate	KOCN	81.11	0.0032444		2.00E-08
	dmso	C2H6OS	78.13	0.0062504	0.05682181818	4.00E-08
	dmsp	C5H10O2S	134.967	0.01079736		4.00E-08
	L-glycine	C2H5NO2	75.07	0.07507		0.0000005
	nucleotides (dNTPs)	-	-	-	0.01	0.00000001
	galactose	C6H12O6	180.16	0.18016		0.0000005
	lactose monohydrate	C12H24O12	360.31	0.36031		0.0000005

	A	B	C	D	E	F
	glyoxylic acid sodium monohydrate	C2H3NaO4	114.03	0.11403		0.0000005
	tmao	C3H9NO	75.11	0.07511		0.0000005
	L-alanine	C3H7NO2	89.09	0.08909		0.0000005
	L-asparagine	C4H8N2O3	132.12	0.13212		0.0000005
	L-aspartic acid	C4H7NO4	133.1	0.1331		0.0000005
	L-cysteine hydrochloride	C3H8ClNO2S	157.62	0.17563		0.0000005
	L-glutamic acid	C5H9NO4	147.13	0.14713		0.0000005
	L-proline	C5H9NO2	115.13	0.11513		0.0000005
	L-serine	C3H7NO3	105.09	0.10509		0.0000005
	sodium alginate	C6H9NaO7	216.12	0.21612		0.0000005
	catalase	C9H10O3	2,000-5,000 U/mg	0.000542857		10 U
	glucosamine	C6H13NO5	221.21	0.22121		0.0000005

Phosphate Mix (1,000x)

	A	B	C	D	E	F
	Compound	**Chemical Formula**	**FW**	**g/100mL**	**mL/100mL**	**Final M**
	ortho phosphonate	C3H9O3P	124.076		0.002167	0.0000002
	potassium phosphate monobasic	KH2PO4	136.09	0.068045		0.000005

Fatty Acid Mix (2,000,000x)

	A	B	C	D	E	F
	Compound	**Chemical Formula**	**FW**	**g/100mL**	**mL/100mL**	**Final M**
	octanoic acid	CH3(CH2)6COOH	144.21		15.84725275	0.0000005
	decanoic acid	CH3(CH2)8COOH	172.26	17.226		0.0000005
	isobutyric acid	(CH3)2CHCO2H	88.11		9.274736842	0.0000005
	butyric acid	CH3CH2CH2COOH	88.11		9.140041494	0.0000005
	valeric acid	C5H10O2	102.13		10.87646432	0.0000005
	ethanol	CH3CH2OH	46.068		54.86150459	0.000004698025433

Inorganic Nitrogen (2,000x)

	A	B	C	D	E
	Compound	**Chemical Formula**	**FW**	**g/100mL**	**Final M**
	sodium nitrate	NaNO3	84.99	0.645924	0.000038
	sodium nitrite	NaNO2	69	0.0276	0.000002
	ammonium chloride	NH4Cl	53.49	0.05349	0.000005

Iron and NTA Mix (1,000x)

	A	B	C	D	E
	Compound	**Chemical Formula**	**FW**	**g/100mL**	**Final M (nM)**
	iron (II) sulfate heptahydrate	FeSO4 × 7H2O	278.01	0.0028	101

	A	B	C	D	E
	nitrilotriacetic acid disodium salt	NTA NA2 salt	235.1	0.0081	345

Stock Preparation

1 Prepare the following stocks prior to medium assembly.

1.1 Iron mix (1,000x)
Trace Metals (100,000x)
Vitamins (100,000x)
AA mix (500x)
Misc. mix (20,000x)
Phosphate mix (1000x)
Inorganic Nitrogen mix (2,000x)
Fatty Acid mix (2,000,000x)

1.2 Filter sterilize stocks using 0.2 µM PES filter.

Base Salts

2 Using an acid-washed and autoclaved one Liter screw-top Pyrex bottle, combine basic salts in 997.4095 mL MilliQ-filtered water. (stock volumes subtracted)

2.1 Mix continuously with stir bar or invert the tightly capped bottle.

Each chemical should be fully dissolved before next addition.

Stock Addition

3 Stocks should be added in a Biosafety cabinet or hood with appropriate ventilation.

Swirl or invert media between stock additions.

3.1 Add (per Liter):
10 µL Trace Metal Mix
10µL Vitamin Mix
20 µL Amino Acid Mix
50 µL Misc. Mix
500 µL Inorganic Nitrogen Mix
0.5 µL Fatty Acid Mix

1mL Iron Mix
1mL Phosphate Mix

4 When in the biosafety cabinet or laminar fume hood filter-sterilize medium using a **0.2 µm filter** into another sterilized one Liter Pyrex bottle.

5 pH and record.

Media can be stored at room temp (~25 C°) in the tightly closed Pyrex bottle.

Protocol references

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