Media Component Sheet: OSM03_01

ASW_m – Modified Artificial Seawater (with additional ions)

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General remarks:

The medium is based on artificial seawater as published in Wilson et al., 1996.[1]

Changes to the original medium composition have been made in terms of

- Additional trace metals used by Myers & Nealson[2] and Tang et al.[3]
- Due to the additional introduction of Fe^{2+} (included in the trace metals noted above), the total molar iron concentration is doubled with respect to ASW.
- If desired, ammonium ions are introduced as NH_4Cl. The original ASW only contains NO_3^- as nitrogen source. In this recipe, the total nitrogen concentration is doubled with respect to ASW.
- The Mn^{2+} in ASW was completely removed; instead, Mn^{2+} is added from an individual stock solution.

This medium cannot/should not be stored once it is prepared in its full form. If storage is needed/desired, the medium should be prepared up to either of the following stages and stored as a "basis medium":

- the 2x ASW basal salt solution (see Part B, section B1)
- after addition of the trace metal mixture and additional supplements as noted in Part A, section A3

PART A: General Protocol (for 1L media):

A1. Prepare 500 ml (or start with) basal salt solution (section B1). NOTE: Additional volume comes from the additives in sections A2 (1 ml), A3 (3 ml) and A4 (various). Compensate for this by lowering volume in Basal Salts (B1) or compensate when diluting the 2x basal salts to the 1x culture medium.

A2. Add 1ml of 1,000x Trace Metal Solution (section B2)

A3. Add each 1ml from individual 1,000x stocks (section B3) of the following

- $Fe^{III}Cl_3$ in 10 mM HCl
- 10 mM NaOH
- EDTA-solution

At this stage, the solution can be stored. If desired, label flask with initials, date, pH, and redox potential (measured with OPR-probe).

A4. If desired, add from stocks (in water; various concentrations; see sections B3&4)

- $Fe^{II}Cl_2$
- NH_4Cl
- $MnCl_2$
- carbon sources (e.g. prepared as 4x concentrated solutions)

A5. When starting out from 500 ml basal salts as outlined here, add finally water to achieve 1 l of medium.

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A6. IMPORTANT NOTE: Strongly redox-active trace metals like Fe^{2+} must be produced freshly before their use (slight yellow colour indicates oxidation after storage). Additionally, Fe^{2+} cannot be autoclaved (oxidation/precipitation) and must be filter-sterilized. The same is recommended for NH_4Cl (ammonium-vapour upon heating likely, hence concentration-loss), which is convenient since a high-concentrated (1 mol/l) stock can be prepared.

PART B: Media Component Solutions:

B1. Basal salts – 2x concentrated stock solution.
Weigh out each salt below and dissolve fully in 500 mL (for 2x-concentrated stock solution) of Milli-Q H_2O. Adjust pH to 8.0 with appropriate NaOH/HCl standard solutions (e.g. 1M).

Salt	**Molar Weight (g/mol)**	**g for 500 ml (of 2x concentrated stock solution)** (= g in 1 L of 1x medium)	**mol/l in final 1x medium**
NaCl	58.44	25.00	$4.28 * 10^{-1}$
KCl	74.55	0.50	$6.71 * 10^{-3}$
$NaNO_3$	84.99	0.75	$8.82 * 10^{-3}$
$K_2HPO_4 * 3 H_2O$	228.20	0.03	$1.31 * 10^{-4}$
$MgSO_4 * 7 H_2O$	246.50	3.50	$1.42 * 10^{-2}$
$MgCl_2 * 6 H_2O$	203.29	2.00	$9.84 * 10^{-3}$
$CaCl_2 * 2 H_2O$	147.00	0.50	$3.40 * 10^{-3}$
Tris	121.14	***Default ASW was 9.08 mM***	**$1.10 * 10^{-3}$**

B2. Trace metal mixture.
(1000x concentrated stock solution of volume of your choice, note that amounts shown below are for 1L of stock solution, hence they need to be updated accordingly if you use a different volume**).**

Trace Metal	**g/l** in 1,000x stock solution	**mol/l** in Medium
$CuCl_2 * 2 H_2O$	0.0055	$3.20 * 10^{-8}$
$ZnSO_4 * 7 H_2O$	0.2200	$7.65 * 10^{-7}$
$CoCl_2 * 6 H_2O$	0.0400	$1.69 * 10^{-7}$
$Na_2MoO_4 * 2 H_2O$	0.4000	$1.65 * 10^{-6}$
H_3BO_3	2.8600	$4.63 * 10^{-5}$
$NiCl_2 * 6 H_2O$	1.0000	$4.20 * 10^{-6}$
$Na_2WoO_4 * 2 H_2O$	0.0800	$2.43 * 10^{-7}$
$Na_2SeO_3 * 5 H2O$	0.0600	$2.28 * 10^{-7}$

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B3. Additional metals (prepared as individual stocks).
(1000x concentrated stock solutions of volume of your choice, note that amounts shown below are for 1L, hence they need to be updated accordingly if you use a different volume**).**

Trace Metal	**g/l** in stock	**mol/l** in Medium
$Fe^{III}Cl_3$ * 7 H_2O; In 10 mM HCl 10 mM NaOH (for pH compensation)	3	$10.4 * 10^{-6}$
EDTA (disodium salt)	0.5	$1.34 * 10^{-6}$
$Fe^{II}Cl_2$ * 4 H_2O	2	$10.00 * 10^{-6}$
$MnCl_2$ * 4 H_2O	*39.58 (200 mM stock solution)*	*as desired*

NOTE: iron-salts cannot be autoclaved because of oxidation/precipitation. Use sterile-filtration instead!!

B4. Optional additions

1.) If desired, add 8.82 ml of NH_4Cl (from 1 mol/l stock) for litre of 1x medium to achieve final concentration of 8.82 mM (equal to the nitrate concentration)
2.) If desired, add 250 ml for 1 litre of 1x medium from 4-fold concentrated carbon source solution. (For smaller volumes than 1 l, add e.g. 10 ml carbon source for 40 ml 1x ASW+carbon. Here, add only 20 ml of the 2x basal salts, and appropriately lowered volumes of the other supplements.

References

(1) Wilson, W. H.; Carr, N. G.; Mann, N. H. *J. Phycol.* **1996**, *32* (4), 506–516.

(2) Myers, C. R.; Nealson, K. H. *Science* **1988**, *240* (4857), 1319–1321.

(3) Tang, Y. J.; Laidlaw, D.; Gani, K.; Keasling, J. D. *Biotechnol. Bioeng.* **2006**, *95* (1), 176–184.

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NOTE: It has been noticed that due to the inability to autoclave the $FeCl_3$ solution, white flakes can appear in solution often followed by small black particles. At this point of contamination running cultures seem to crash out (growth density does not increase when sub-cultured into fresh media). Therefore, it is advised to take note of this.

Also, flaky material has been noted in the sterile basal salts when the volume in the 2L bottles reaches below 80-90%, this may be due to crystallization or some other chemical process, but this also appears to cause the culturing the fail. It is advised to make fresh media components before this stage is reached if it is

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wished to run a continuous running culture of the species.