

ATCC Medium: 2684 DME/F12-3 Growth Medium (29ppt)

	<u>500ml</u>	<u>1000ml</u>
DME powder (Sigma D5030)	2.1g	4.2g
Ham's F-12 nutrient mix (Sigma N6760)	2.7g	5.4g
200mM L-glutamine [2mM, total final]	2.5ml	5.0ml
1.0M HEPES [25mM, final]	12.5ml	25.0ml
7.5% NaHCO ₃ [7mM, final]	3.2ml	6.4ml
JLP carbohydrates**	5.0ml	10.0ml
Yeast extract ultrafiltrate (Sigma Y4375)	1.0ml	2.0ml
0.5% phenol red [0.00037%, total final]	0.24ml	0.48ml
15‰ SASW*	455ml	910ml
1.0N HCl (for final pH 7.0)	~1.5ml	~3.0ml

1. Dissolve and filter at 0.2µm.

2. Add optional, sterile components (below) aseptically.

FBS [3% v/v final]	15.0ml	30.0ml
Lipid Concentrate 1,000x (Sigma L5146 or equiv.)	0.5ml	1.0ml

Optional antibiotics @ standard concentrations

(*P. marinus maxima*)

penicillin-streptomycin @ 100 U-µg/ml	(1,000 U-µg/ml)
gentamicin @ 50µg/ml	(5,000 µg/ml)
chloramphenicol @ 5 µg/ml	(50 µg/ml)
amphotericin B @ 0.25 µg/ml	(0.25 µg/ml)
cycloheximide @ 0.05 µg/ml	(0.05 µg/ml)
nystatin @ 200 U/ml (~40µg/ml)	(400U/ml), (~80µg/ml)

3. Store medium at 4°C. Re-supplement to 2mM L-glutamine every 60 days.

* 15‰ SASW: 8.5g sea salt (Sigma S9883) in 455ml TCGW. Autoclave @ 121°C, 30 min.

** JLP carbohydrates: 5.0g glucose, 1.0g galactose, 1.0g trehalose in 100ml TCGW. Filter at 0.2µm

TCGW = tissue culture grade water (18MΩ nanopure, or equivalent)

FBS = fetal bovine serum

Medium final osmolality is about 850 mOsm/kg (29.5‰).

Medium pH equilibrated in 27°C air atmosphere is about 7.5.

If pre-mixed 1:1 DME/Ham's F-12 powder is used, adjust L-glutamine, phenol red, etc. supplements.

(Revised from Dungan and Hamilton 1995. *J. Eukaryotic Microbiol.* **42**:379-388)