

## ATCC Medium: 2860 Modified DMEM/F12 Medium

DME powder (Sigma D5030).....	4.2 g
Ham's F-12 nutrient mix (Sigma N6760).....	8.4 g
200mM L-glutamine.....	5.0 ml
1.0M HEPES.....	25.0 ml
7.5% NAHCO <sub>3</sub> .....	6.4 ml
JLP carbohydrates (see below).....	10.0 ml
Yeast extract ultrafiltrate (Sigma Y4375).....	2.0 ml
0.5% phenol red.....	0.48 ml
15% SASW (see below).....	910 ml
1.0N HCl (for final pH 7.0).....	~3.0 ml

Dissolve and filter at 0.2um.

Add optional, sterile components aseptically:

Fetal Bovine Serum.....	30 ml
Lipid Concentrate 1,000x (Sigma L5146 or equiv.).....	1.0 ml

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

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

Medium pH equilibrated in 27C 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.

### **15% SASW**

Sea Salt (Sigma S9883).....	8.5 g
Tissue Culture Grade Water.....	455 ml

Autoclave at 121C.

\*\*Alternatively use any artificial seawater mix (i.e., Crystal Sea Marinemix at 15-20 g/liter).

### **JLP carbohydrates**

Glucose.....	5.0 g
Galactose.....	1.0 g
Trehalose.....	1.0 g
Tissue Culture Grade Water.....	100 ml

### **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)