**Bacteroidetes Media Solutions & Supplements**

**Balch’s Vitamins**

- p-Aminobenzoic acid, 5mg
- Folic acid, 2mg (Sigma, F7876)
- Biotin, 2mg (Sigma, B4501)
- Nicotinic acid, 5mg (Sigma, N4126)
- Calcium pantothenate, 5mg (Sigma, P2250)
- Riboflavin, 5mg (Sigma, R7649)
- Thiamine HCl, 5mg (Sigma, T4625)
- Cyanocobalamin (vitamin B12), 0.1mg
- Thioctic acid (lipoic acid), 5mg

Dissolve vitamins, pH to 7.0, filter sterilize, keep at 4C in dark.

**Amino Acid Solution**

- Alanine, Arginine, Asparagine, Aspartic Acid, Cysteine, Glutamic Acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine

Dissolve 62.5mg each of the 20 essential amino acid to 250 ml (5mg/ml total stock). Filter sterilize, store at room temperature.

**Purine/Pyrimidine Solution**

- Adenine (Sigma, A2786)
- Guanine (Sigma, G11950)
- Thymine (Sigma, T0895)
- Cytosine (Sigma, C3506)
- Uracil (Sigma, U1128)

Dissolve 200mg each into 1L (1mg/ml total stock), pH to 7.0, filter sterilize and store at room temperature.

**Trace mineral Supplement**

- EDTA, 0.5g (Sigma, ED4SS)
- MgSO₄·7H₂O, 3g
- MnSO₄·H₂O, 0.5g
- NaCl, 1g (Sigma, S7653)
- FeSO₄·7H₂O, 0.1g (Sigma, 215422)
- CaCl₂, 0.1g
- ZnSO₄·7H₂O, 0.1g
- CuSO₄·5H₂O, 0.01g
- H₃BO₃, 0.01g (Sigma, B6768)
- Na₂MoO₄·2H₂O, 0.01g
- NiCl₂·6H₂O, 0.02g

Dissolve in 1L, pH to 7.0, filter sterilize and store at room temperature.

**10X Bacteroides Salts (4L Recipe)**

- 544g KH₂PO₄ (Fisher, P284)
- 35g NaCl (Sigma, S7653)
- 45g (NH₄)₂SO₄ (Fisher, A702)

Dissolve and pH to 7.2, filter sterilize in 1L batches. Store at room temperature.
**Minimal Media Supplements**

Vitamin K₃ (menadione, Sigma, M5625) stock: 1mg/ml in ethanol, wrap in foil.
FeSO₄ (Sigma, 215422) stock: 0.4mg/ml in 10mM HCl, filter sterilized
MgCl₂ (Sigma, M8266) stock: 0.1M in water, filter sterilized
CaCl₂ (Sigma, C1016) stock: 0.8% (w/v) in water, filter sterilized
Histidine Hematin (Sigma, H8000 & H3281) solution stock: 1.9mM Hematin in 0.2M Histidine solution, filter sterilized.
This solution is highly insoluble. Add hematin to 1mL 1M NaOH and allow to fully dissolve. Neutralize with 1mL 1M HCl.
Bring to a final volume with the Histidine solution. Filter sterilize, wrap in foil.
Vitamin B₁₂ (Sigma, V2876) stock: 0.01mg/ml in water, filter sterilized, wrap in foil.

I usually make 50 ml batches (250 ml for vitamin B₁₂ because of low concentration). Keep all solutions at 4C.

**Bacteroidetes Minimal Media Recipes**

**1X Minimal Monosaccharides Recipe (250 ml)**

25 ml 10X *Bacteroides* Salts
2.5 ml each: Balch’s Vitamins, Trace Mineral Solution, Purine/Pyrimidine Solution, Amino Acid Solution.
250 ul each: K₃, FeSO₄, CaCl₂, MgCl₂, Hematin stocks
125 ul B₁₂ stock
250 mg L-Cysteine
250 mg each*: Glucose, Galactose, Xylose, Fructose, Mannose, N-Acetyl Glucosamine

Bring to volume with distilled water, pH to 7.2, filter sterilize

*Most Bacteroidetes will grow on glucose alone. However, several monosaccharides are used here in case an organism grows poorly or is incapable of growth solely on glucose.

**2X Minimal Media-no Carbon (50 ml)**

10ml 10X *Bacteroides* Salts
1 ml each: Balch’s Vitamins, Trace Mineral Solution, Purine/Pyrimidine Solution, Amino Acid Solution
100 ul each: K₃, FeSO₄, CaCl₂, MgCl₂, Hematin-Histidine stocks
50 ul B₁₂ stock
100 mg L-Cysteine

Bring volume to 50 ml with distilled water, pH to 7.2, filter sterilize. This will be mixed with a 2x (usually 10mg/ml) carbon source stock for a 5mg/ml final carbon source minimal media. Exceptions are (1) Neutral Mucin O-glycans and (2) Rhamnogalacturonan II which should be at 20mg/ml stocks and thus a 10mg/ml final media concentration.

**Note:** If bacteria are still growing poorly, beef extract (Sigma, B4888) can be added to 5mg/ml final into minimal media (10mg/ml in 2X minimal that will be 5mg/ml final when added to carbon source). This must be added prior to media filter sterilization or added as a sterile stock. A calculated negative control must be done in any growth experiments to determine if beef extract is being used as a carbon source. If so, this growth must be subtracted from carbon source growth.

**Custom Chopped Meat Recipe**

2.5g Beef Extract
7.5g Pancreatic digest of casein
1.25g Yeast Extract
1.25g K₂HPO₄
250mg Cysteine
250 mg each: Glucose, Galactose, Xylose, Fructose, Mannose, N-Acetyl Glucosamine
250ul each: K₃, B₁₂ stocks
1ml Hematin-Histidine stock
2.5ml each: Balch’s Vitamins, Trace Mineral Solution, Purine/Pyrimidine Solution, Amino Acid Solution

Bring to 250 ml, pH to 7.2 and filter sterilize.

This media cannot be autoclaved. So, if you need agar plates then just double the concentration of everything and mix with a 2X (30mg/ml) agar solution. You may also add in any antibiotics desired.