BG(N+) Plates // BG(N+) Salts

Ingredients: Bacto agar, BG#1-BG#8, $Na_2S_2O_3$

Special Instructions: Pour the plates in the hood. Be careful, while pouring, to avoid bubbles in the media. If there are bubbles in the plate, use the flame to pop them. Flame the lip of all bottles and flasks often. There should be 40 ml in each plate.

Amount Making: 1000 ml = 1 sleeve = 25 plates

Recipe:

- **Agar**:
  - Add 15 g Bacto agar to 500 ml pol H_2O in a 1L flask or 1L bottle
  - Autoclave the agar for 40 min (also can be autoclaved along with the salts)
- **Salts**:
  - Add 475 ml pol H_2O to a 500 ml autoclavable bottle
  - Add 10 ml BG#1 to the bottle
  - Add 1 ml BG#2-BG#8 (in numerical order) to the bottle
  - Autoclave the salts for 30 min (also can be autoclaved along with the agar)
- Put agar and salts in 55ºC waterbath immediately after they comes out of the autoclave
- Pour the plates, after they sit 1 hour in the waterbath:
  - Add 10 ml $Na_2S_2O_3$ with a sterile pipette to the salts
  - Add any antibiotics to the salts at this time
  - Mix solution by swirling
  - Add salts to the agar, then mix well so the mixture is homogeneous
  - Measure with a graduated cylinder 40 ml of media to pour into each plate

Storage: Special:

- Leave to solidify (~3 hours) then they can be used

Reference / Notes: The salts can be made up at a separate time before the agar and kept at room temperature after they are autoclaved, then put in the waterbath when you need them. Since we add 15 g of agar for 1000 ml of media, the plates we make are 1.5% agar. Having the salts and agar sit 1 hour in the waterbath is so they are at a safe temperature to add antibiotics to, but as soon as they are at the correct temperature the plates can be poured.

100mM Sodium Thiosulfate ($Na_2S_2O_3$)

Ingredients: $Na_2S_2O_3$ x $5H_2O$ (or $Na_2S_2O_3$, anhydrous)

Special Instructions:, If you use $Na_2S_2O_3$ x $5H_2O$ then add 12.4 g, but if you use $Na_2S_2O_3$, anhydrous then add 7.9 g. **Filter Sterilize; DO NOT autoclave.**

Amount Making: 500 ml

Recipe:

- Add [See: Special Instructions] $Na_2S_2O_3$ ~350 ml deionized H_2O in a 500 ml flask
  - Currently: 12.4 g
- Raise the volume to 500 ml
- Filter sterilize through a bottle top filter into a 500 ml autoclavable bottle
  - If making small aliquots, put 50 ml into 5 autoclavable bottles and only make half of Recipe amount (250 ml)

Storage: 4°C Fridge

Updated by Pichaporn Kanjanakantorn (1/25/17)

Reference / Notes: none

BG(N+) Bottles

Ingredients: BG#1-8
Special Instructions: Add the BG#1-8 in numerical order. *For BG(N-) bottles, just leave out BG#1.*
Amount Making: 1 L (10 x 100 ml)
Recipe:

- Add 10 ml BG#1 to ~900 ml deionized H_2O in a 1 or 2 L flask
- Add 1 ml BG#2-8 in numerical order to the same flask
- Adjust the pH to 7.5 with HCl
- Raise the volume to 1 L, then mix well so the mixture is homogeneous
- Add 100 ml to 10 (125 ml) autoclavable bottles
- Autoclave 30 min

Storage: Room Temperature
Reference / Notes: none

	2L	**3L**	**4L**	**6L**	**8L**
BG#1	20 ml	30 ml	40 ml	60 ml	80 ml
BG#2-9	2 ml	3 ml	4 ml	6 ml	8 ml

BG(N+) Flasks

Ingredients: BG#1-8
Special Instructions: Add the BG#1-8 in numerical order. *For BG(N-) flasks, just leave out BG#1.*
Amount Making: 1 L (10 x 100 ml)
Recipe:

- Add 10 ml BG#1 to ~900 ml deionized H_2O in a 1 or 2 L flask
- Add 1 ml BG#2-8 in numerical order to the same flask
- Adjust the pH to 7.5 with HCl
- Raise the volume to 1L, then mix well so the mixture is homogeneous
- Add 100 ml to 10 (250 ml) flasks
- Top flasks with cotton plugs (only good ones) and caps
- Autoclave 30 min

Storage: Room Temperature
Reference / Notes: We use to rinse out the flasks with H_2O to get rid of any detergent/residue in the flasks, as Cyanos are very sensitive to that, but don't anymore as the dishwasher does a good job of removing excess detergent.

	2L	**3L**	**4L**	**6L**	**8L**
BG#1	20 ml	30 ml	40 ml	60 ml	80 ml
BG#2-9	2 ml	3 ml	4 ml	6 ml	8 ml

All BG-11 stock solutions are kept in the refrigerator.

BG#1

ingredients: $NaNO_3$ [Sodium nitrate]
Special Instructions: **Use the small autoclave for sterilization**
amount making: 500 ml of 150 g/L (100x)
recipe:

- Add 75 g $NaNO_3$ to ~300 ml purified H_2O in a 1 L flask.
- Stir till dissolved.
- Raise volume to 500 ml in a graduated cylinder.
- Transfer solution to a 500 ml autoclavable bottle.
- Autoclave for 30 min.

BG#2

ingredients: $CaCl_2$ x $2H_2O$ [Calcium chloride, dihydrate]
special instructions: These measurements are for this formula ($CaCl_2$ x $2H_2O$) only. They must be recalculated for a different formula.
amount making: 500 ml of 36 g/L (1000x)
recipe:

- Add 18 g $CaCl_2$ x $2H_2O$ to ~300 ml purified H_2O in a 1 L flask.
- Stir till dissolved.
- Raise volume to 500 ml in a graduated cylinder.
- Transfer solution to a 500 ml autoclavable bottle.
- Autoclave for 30 min.

BG#3

ingredients: $C_6H_9FeNO_7$ [Ferric ammonium citrate] (6 g/L)
$C_6H_8O_7$ [Citric acid] (6 g/L)
special instructions: **Filter Sterilize; DO NOT autoclave.** This solution can turn from a golden brown to a greenish color over time.
amount making: 250 ml of 6 g/L each (1000x)
recipe:

- Add 1.5 g Ferric ammonium citrate to ~200 ml purified H_2O in a 500 ml flask.
- Add 1.5 g Citric Acid to the flask.
- Stir till dissolved.
- Raise volume to 250 ml in a graduated cylinder.
- Filter sterilize into a 250 ml sterile bottle (we wrap in foil for unknown historical reasons)

Updated by Pichaporn Kanjanakantorn (1/25/17)

BG#4

ingredients: EDTA x Na_2 [EDTA disodium]
special instructions: If you use the **dihydrate**, you must use 1.1 g of EDTA disodium, dihydrate.
amount making: 500 ml of 1 g/L (1000x)
recipe:

- Add 0.5 g Disodium EDTA to ~400 ml purified H_2O in a 1 L flask.
  - Currently (since 7/11/16): 1.1g of EDTA disodium, **dihydrate**
- Stir till dissolved.
- Raise volume to 500 ml in a graduated cylinder.
- Transfer solution to a 500 ml autoclavable bottle.
- Autoclave for 30 min.

BG#5

ingredients: K_2HPO_4 [Potassium phosphate, dibasic]
special instructions: none
amount making: 500 ml of 40 g/L (1000x)
recipe:

- Add 20 g K_2HPO_4 to ~400 ml purified H_2O in a 1 L flask.
- Stir till dissolved.
- Raise volume to 500 ml in a graduated cylinder.
- Transfer solution to a 500 ml autoclavable bottle.
- Autoclave for 30 min.

BG#6

ingredients: $MgSO_4$ x $7H_2O$ [Magnesium sulfate, heptahydrate]
special instructions: This recipe is only for this formula of $MgSO_4$ x $7H_2O$. If you use $MgSO_4$ (anhydrous) then add 18.05 g instead of 37.5 g.
amount making: 500 ml of 75 g/L (1000x)
recipe:

- Add 37.5 g $MgSO_4$ x $7H_2O$ to ~400 ml purified H_2O in a 1 L flask.
- Stir till dissolved.
- Raise volume to 500 ml in a graduated cylinder.
- Transfer solution to a 500 ml autoclavable bottle.
- Autoclave for 30 min.

BG#7

ingredients: Na_2CO_3 [Sodium carbonate]
special instructions: **Filter Sterilize; DO NOT autoclave**
amount making: 500 ml of 20 g/L (1000x) (PCC recipe uses 2x original or 40 g/L)
recipe:

- Add 10 g Na_2CO_3 to ~400 ml purified H_2O in a 1 L flask.
- Stir till dissolved.

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- Raise volume to 500 ml in a graduated cylinder.
- Filter sterilize into a 500 ml sterile bottle.

BG#8

ingredients: H_3BO_3 [Boric acid]
$MnCl_2$ x $4H_2O$ [Manganese(II) chloride, tetrahydrate]
$ZnSO_4$ x $7H_2O$ [Zinc sulfate, heptahydrate]
$Na2MoO_4$ x $2H_2O$ [Sodium molybdate, dihydrate]
$CuSO_4$ x $5H_2O$ [Copper(II) sulfate, pentahydrate]
$Co(NO_3)_2$ x $6H_2O$ [Cobalt(II) nitrate, hexahydrate]

special instructions: **Filter Sterilize; DO NOT autoclave**
amount making: 500 ml (1000x)
recipe:

- To ~400 ml purified H_2O in a 1 L flask.
- Add 1.43 g H_3BO_3.
- Add 0.905 g $MnCl_2$ x $4H_2O$.
- Add 0.111 g $ZnSO_4$ x $7H_2O$.
- Add 0.195 g $Na2MoO_4$ x $2H_2O$.
- Add 0.0395 g $CuSO_4$ x $5H_2O$.
- Add 0.0245 g $Co(NO_3)_2$ x $6H_2O$.
- Stir till dissolved.
- Raise volume to 500 ml in a graduated cylinder.
- Filter sterilize into a 500 ml sterile bottle.

Final Components of BG-11 per 1 Liter:

$NaNO_3$	[BG#1]	= 1.5 g
$CaCl_2$ x $2H_2O$	[BG#2]	= 0.036 g
Citric acid	[BG#3]	= 6.0 mg
Ferric ammonium citrate	[BG#3]	= 6.0 mg
EDTA x Na_2	[BG#4]	= 1.0 mg
K_2HPO_4	[BG#5]	= 0.04 g
$MgSO_4$ x $7H_2O$	[BG#6]	= 0.075 g
Na_2CO_3	[BG#7]	= 0.02 g
Trace Metals	[BG#8]	= 1.0 ml