**Procedure**

1. Prepare minimal+Arg or TARG media and autoclave as normally would.
2. During autoclave cycle, measure 40 mg carbendazim (for 1 L) in fume hood. Take special precaution when handling carbendazim – use proper PPE including gloves, goggles/fume hood, to prevent entry into respiratory system.
3. Dissolve carbendazim in 0.5-1 ml dimethyl-formamide (DMF).
4. Following liquid autoclave cycle, remove and place on stir plate to cool, stirring about 45-70 min.
5. Once media is cool enough to hold indefinitely, use sterile technique to add antibiotics and fungiside in the laminar flow hood. Return to stirring plates to mix well (~2-5 min). Pour plates and allow to cool 4-16 hrs, allow some ventilation to limit condensation.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Chemical** | **Protects Against** | **Stock Concentration** | **Final Concentration** | **Solvent** | **Volume of Stock per 1L** |
| Ampicillin | Broad spectrum – gram +/- bacteria | 100 mg/ml | 500 ug/ml | Water | 5 ml |
| Cefotaxime | Broad spectrum – gram +/- bacteria | 50 mg/ml | 100 ug/ml | Water | 2 ml |
| Carbendazim | Broad spectrum – fungi | 80 mg/ml | 40 ug/ml | DMF | 0.5 ml  |

**Additional Comments**:

Since the media now contains these chemicals, it’s best to wear gloves while pouring and handling these plates. When preparing aliquots for these antibiotics, remember to filter sterilize before aliquoting. I don’t filter sterilize the carbendazim because the DMF was a bit more viscous and thought it might clog the filter – has yet to negatively affect sterility (so 2+ years not an issue). Carbendazim won’t effectively go into solution if added directly to media, must first dissolve in DMF.

**Adapted from:**

Kan, Yinan, and Junmin Pan. "A One-Shot Solution to Bacterial And Fungal Contamination In The Green Alga *Chlamydomonas Reinhardtii* Culture By Using An Antibiotic Cocktail1." *Journal of Phycology* 46.6 (2010): 1356-358. Web.

Mahan, Kristina, Obed Odom, and David Herrin. "Controlling Fungal Contamination in *Chlamydomonas* *Reinhardtii* Cultures." *BioTechniques Biotech.* 39.4 (2005): 457-58. Web.