Antifungal compounds for media

Examples of reagents used in our lab:

- Cykloheximide 100 mg/L
- Nystatin 50 mg/L

Note :

- These are highly toxic compounds.
- Both compounds have limited solubility in water.
- These compounds cannot be autoclaved and must therefore be sterile filtered (0,22 µm).
- Both are light sensitive compounds – store dark.

Procedure:

1. Check up safety rules. Were face mask, glasses and gloves. For weighing, use an analytical balance with protective glass walls.

2. Use a small glass beaker or similar.

3. <u>If you use cykloheximide (CH):</u>

   - Add distilled water and EtOH/MeOH (solubility of CH in water 20-25 mg/l (add e.g. 200 µl water and 800 µl 96 % EtOH/MeOH)*.
   - Vortex until the solution has dissolved.
   - Sterile filtrate the CH solution directly into your medium vessel (use the lid to protect the bottle opening to minimize exposure to air). Mix carefully the medium before you pour the plates/aliquot into tubes.

4. <u>If you use nystatin,</u>
   - Add DMF (dimethylformamide) or formamide (freely soluble).**
   - Vortex until the solution has dissolved.
   - Do not sterile filter (especially if you used DMF to dissolve nystatin, DMF may destroy the filter and the holder!). Just add the dissolved solution into the agar bottle. Mix carefully the medium before you pour the plates/aliquot into tubes.

*CH is also soluble in cloroform, methanol, and acetone; moderately soluble in isopropanol, n-butanol and amyl acetate; very slightly soluble in carbon tetrachloride and the saturated hydrocarbons. Note however that some of these reagents may destroy plastic vessels – use therefore glass vessels.

**Nystatin is only minimally soluble in water- is freely dissolved in DMF or formamide and to some extent to other compounds like DMSO, methanol etc.Note however that some of these reagents may destroy plastic vessels – use therefore glass vessels.