ATCC medium: 2680 HD medium

For Broth
Peptone ……0.5 g
Glucose……0.1 g
Yeast extract……0.25 g
MES…….1.95 g
Distilled water for liquid medium …..1000 ml

Adjust pH to 5.5 with NaOH/HCl. Dispense and autoclave @ 121C

For Agar

Solution A
Peptone ……0.5 g
Glucose……0.1 g
Yeast extract……0.25 g
MES…….1.95 g
Distilled water for solid medium…….500 ml

Solution B
*****Washed agar (w/ double distilled water) or Noble Agar (Difco) 15 g
Double distilled water…….500 ml

Adjust pH to 5.5 with NaOH/HCl

Sterilize solutions A and B separately by autoclaving at 121˚C for 15 minutes. For solid medium combine solution A with solution B at 50 ^{o}C.

***Background**
Agar is a natural product, and different sources and batches are purified to varying degrees. Difco Bacto-agar contains impurities that may inhibit or may provide supplements that interfere with scoring of certain mutants (Ryan *et al.* 1943). When Bacto-agar is suspended in water, impurities are abundant enough to color the supernatant.
While unwashed agar is completely satisfactory for routine work, the impurities become a problem in special situations, as when growth rate is being determined or in experiments with mutants that have fastidious requirements. With unwashed agar, recognition of specific nutritional requirements may in some instances be sharpened by the addition of analogs or antagonists that compete for uptake. For example, the requirement for biotin is sharpened by adding avidin or egg white. However, an appropriate antagonist may not be available or use of an antagonist may not be feasible or desirable. Purified, washed agar provides an alternative.

Procedure
The following is from Ryan (1950):
"Since Bacto-agar (powdered) contains inhibitory substances and vitamins and amino acids (Ryan *et al.* 1943), it should be washed before use. In a 5-gal. Carboy with a 3-holed stopper introduce 1 pound of agar, feed distilled water through a glass tube reaching to the bottom, and shake the vessel vigorously. After

settling for ½ hour the agar half fills the carboy. A third glass tube, reaching almost to the agar, siphons off the supernatant. After repeating the washing 15 times (within 2 days to avoid biologic contamination), collect the agar by filtering with suction through coarse paper in a large Buchner funnel, place in a battery jar, cover with 95% alcohol and let stand for about ½ hr. Then filter, resuspend in 95% alcohol for about 4 hr and filter again. Bring the same volume of 95% alcohol to boiling, introduce the agar and again bring to boiling. Then filter the suspension until most of the alcohol is extracted (about ½ hr). Spread the white fluffy agar on aluminum foil to dry. Do not use heat. The product should be powdery, white and clean. If lumpy, it can be made granular by dry agitation in a Waring blendor."

The following description is from Griffith's *How to make heterokaryons*:

"For most routine work, regular agar is adequate. However, it does contain low levels of nutrients, and these can be a problem in some cases. For leaky forcing markers, or for cleaner results, it is best either to use commercially purified agar or to "wash" some regular agar.

Agar is washed as follows. Cover some agar powder with tap water and let it stand overnight. Decant and add fresh water a total of three times over three days. Next suspend overnight in distilled water. Decant and put the agar into a nylon grape-pressing bag. Squeeze out as much water as possible by hand. If a wine press is available, squeeze the bag in the press. Resuspend in acetone overnight in a fume hood. Wearing rubber gloves, squeeze again manually or if possible in the press. Spread the squeezed-out agar on trays in the fume hood to dry, which takes several days. Stir it occasionally. Grind the dried agar in a food blender To make a powder that will more easily dissolve when making up medium."

References

Ryan, F.J. 1950. Selected methods of Neurospora genetics. Methods Med. Res. 3: 51-75