Culture in Place - Media Loading Methods for MicroFunnel™ Filter Funnels

Method A: Transferring the Membrane

1. Assemble the Manifold, Vacuum Flask Trap, Vacuum pump, and Vacushield™ filter.
2. Remove the sterile funnel from the bag. Place funnel directly onto the Pall Life Sciences aluminum manifold. If you do not have a Pall Life Sciences aluminum manifold, insert funnel adapter into no. 8 stopper, then place stopper/adapter assembly into manifold or flask.
3. Optional prewet. Remove cover without touching the inner surface and rinse inside walls of funnel and wet out membrane surface. Replace cover and turn on vacuum to draw rise fluid through. Turn off vacuum.
4. Remove cover without touching the inner surface, and pour sample into the funnel. Replace cover.
5. Turn on vacuum and draw the sample through the filter. Filter the entire sample first, then rinse the inside walls of the funnel with 60 mL of sterile buffer solution.
6. Remove the lid then hold the Microfunnel filter unit with one hand and gently squeeze the funnel cylinder near the top. Carefully remove the funnel cylinder from the base.
7. Dip the tips of the stainless steel forceps into alcohol and flame to sterilize.
8. Insert forceps and remove the membrane from the base leaving behind the support pad.
9. Place the membrane filter on a prepared agar plate or Petri dish/absorbent pad combination soaked with broth.
10. Invert and incubate at the specified temperature and time.
11. Count the colonies using a binocular microscope at 15X.
12. Discard the Petri dish following appropriate federal, state and local guidelines for disposal of biohazardous material.
13. The Microfunnel filter unit is designed for SINGLE USE ONLY.

Method B: Top Load Media and collapse to Petri dish on the Manifold

1. Filter and rinse the sample as described above in Method A, steps 1-5.
2. Allow vacuum to draw for an additional 15-20 seconds after final rinse fluid has evacuated the funnel. This helps remove as much fluid from the pad as possible.
3. Turn off vacuum source. Do not remove funnel from manifold. Do not remove cylinder from base.
4. Carefully dispense the ampoule media over the surface of the membrane and allow residual vacuum to draw the media through the membrane and into the pad to displace the residual rinse fluid. If there is no residual vacuum in the manifold, then momentarily apply enough vacuum to draw the media through the membrane and into the pad.
5. Remove the lid and remove the cylinder with a simple squeeze.
6. Place lid securely onto base and remove from manifold.
7. Plug the base.
8. Invert and incubate.

Method C: Top Load Media, tap off excess

1. Filter and rinse the sample as described above in Method A, steps 1-5.
2. Allow vacuum to draw for an additional 15-20 seconds after final rinse fluid has evacuated the funnel. This helps remove as much fluid from the pad as possible.
3. Turn off vacuum source. Do not remove funnel from manifold. Do not remove cylinder from base.
4. Carefully dispense the ampoule media over the surface of the membrane and allow residual vacuum to draw the media through the membrane and into the pad to displace the residual rinse fluid. If there is no residual vacuum in the manifold, then momentarily apply enough vacuum to draw the media through the membrane and into the pad.
5. Remove funnel from the manifold.
6. Plug the base.
7. Remove the lid and remove the cylinder with a simple squeeze.
8. Tap off excess media by holding the base almost perpendicular to the countertop and shake or tap excess onto absorbent padding or towel. Be sure not to disturb the analysis by inverting and tapping or otherwise causing the membrane and pad to fall out of the base.
9. Place lid securely onto base to form a Petri dish for incubation.
10. Invert and incubate.