

Precoating and Pouring Agar Slides

Precoating

0.2% Agar : Agar (Oxoid): 0.2 g

H₂O to 100 ml

Autoclave 15' at 270°F (Use a 250 ml black screw top flask in the autoclave)

(20 mg Na Azide may be added as a preservative)

Slides and plates which are to be precoated are first washed in 7X detergent & rinsed w/dH₂O. Just before coating, spray slides liberally with 95% Ethanol and wipe with Kim wipes.

Pour 0.2% Agar into a wide mouth beaker and place in water in electric pan.

Set temperature control to 200-250°F. Always keep temperature of agar above 150°F.

Using long tongs hold slides and dip into beaker with agar. Dip the slides quickly so that agar will not clump. After dipping the slides lean onto the side of a drying basket. ***Note large plates probably can not be dipped--one must use a small beaker and pour the agar all over the plate. Pouring, as dipping must be done quickly. Always avoid dust!!

Once slides have been dipped they are dried in the plastics dryer in the kitchen overnight.

After drying cover slides with a lint free cloth or saran wrap to protect from dust.

Pouring the Agar

2.0% Agar: Agar(Oxoid): 2.0 g

H₂O to 100 ml

Autoclave for at least 15 minutes at 275°F

Meanwhile put 100 ml of 2X Barbitol Buffer (pH 8.2, 0.1M) into 400ml beaker and then into the 65°C water bath--this is simply to warm the buffer up before adding the hot agar to it.

Pouring Agar Slides-Continued

Remove agar from autoclave.

Mix 100ml of the agar with 100 ml of the warm Barbital Buffer. (Now you have a 1% agar solution in 1X Barbital buffer (0.05M).)

Put this agar solution into a wide mouth beaker and put the beaker into the electric pan which has some warm water in it. The temperature gauge on the pan should be set at 200°F.

Lay the precoated slides on a flat surface (check with level).

Pour the proper amount of agar onto each slide using a pipet.

Small Microscope slides (7.5 x 2.5 cm): 3 ml/slide

Standard Immuno Slides (7.5 x 5.0 cm): 6 ml/slide

Medium sized Plates (20 x 10cm): 32 ml/plate

Large Plates (16 x 16 cm): 42 ml/ plate

Let the agar harden at least one half hour. Then place slides on a piece of filter paper which has been dampened with distilled water and place slides & paper in plastic dish. The larger plates are simply placed against the wire racks in the tupperware container.

Store all plates and slides in tupperware containers--always make sure that damp paper towels are in the bottom of the container and that the containers are tightly sealed!!!