

Protocol graciously provided by: Dr. Mariano Viapiano, Viapiano Lab protocols (© 2006-2014)

B. Preparation of agar plates to make aggregates of adherent cells

1. In a 50 ml conical tube dissolve 0.4g agarose GPG/LE (American Bioanalytical AB00972) in 40 ml high- glucose DMEM (Invitrogen). Use DMEM without serum or antibiotics.
2. The agarose won't dissolve completely. Put the mixture in a boiling water bath and watch until agarose is completely dissolved (keep tube loosely covered).
3. Let the dissolved agar cool down to ~50 °C (otherwise it will break the filter) and filter-sterilize in a culture hood using a 0.22 µm filter (Millipore Steriflip).
4. Quickly pipette dissolved agar into 35 mm culture dishes (1.5 ml/dish), you need to do this quickly before the agar solidifies.
5. Half-cover the dishes and leave them in the hood until the agar solidifies.
6. Wrap the plates in saran-wrap (4-5 plates together) and store at 4 °C until use (they last ~3 weeks before the agar starts cracking).