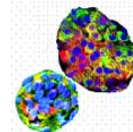


SOP



Title:	Preparing slides for immunostaining of clusters				
Protocol #:	1.5	Submitted:	050610	Approved:	200610
Category:	Cyto	Author(s): ¹	AAH	Checked by:	AAH

Reagents:

1. Any fibroblast cells line / HEK cells
2. Distilled water

Equipment

1. Slides / coverslips
2. Inverted microscope
3. CO₂ incubator
4. pipettes

Reagent Setup

1. This is a protocol that will help you to adhere the cell clusters generated in vitro / islets on to slides / coverslips for immunostaining / FISH. In our experience, human islets do not stick well on to slides after cytopspining. There are commercially available glues for cell adhesion but the protocol provided here allows easy, reliable and quick adhesion of cell clusters / spheroids / embryoid bodies / neurospheres that can be then used for immunostaining or FISH.
2. Grow fibroblast cell line on glass slides / labtek chamber slides / coverslips (kept in 6 well plates). Cells should be seeded at a density such that they become confluent on the day when you want to fix your cell clusters / neurospheres / islets.

Procedure:

1. On the day when you want to fix your cell clusters / spheroids, take a look at the slides / labteks / coverslips on which the fibroblast cell line was seeded. These cells deposit extracellular matrix in ~2 days of culture.
2. On the day of seeding, take out the medium from the fibroblast cell culture and add distilled water to the plate. Wash the plate once to remove any traces of media, and incubate the plate with distilled water for ~15 minutes. This will allow hypotonic lysis of cells so that all of the cellular contents are removed. ECM will remain on the plates
3. Once all the cells are lysed by hypotonic shock, confirm the same by observing the slide on an inverted microscope and then drain off any excess water from the slides / coverslips / labtek chambers.

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4. Now, seed the cell clusters / embryoid bodies / spheroids / cell aggregates on to the slides prepared above (in #3) in minimal volume of serum containing medium. Place these slides in the incubator at 37°C for 30-40 minutes. Most aggregates will adhere in around 20 minutes and these plates can be removed in this time. After removal, fix the cell aggregates that have adhered on to the slides immediately by using 4% fresh Paraformaldehyde.
5. Proceed with immunostaining as usual.