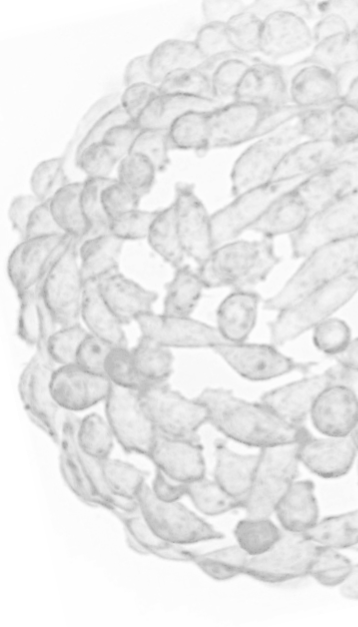

# Immunohistochemical Staining of Cells on the GEM v1.0

Here is a general method for fixing and labeling cells cultured on the GEM using a fluorescent secondary antibody for detection. Values such as time and number of washes are general and may vary for your antibody, sample thickness and preparation.

1) Begin by adding a blocking solution to 1.5 mL snap top tubes. The blocking solution can be what you usually use for block or a 1% BSA solution.
2) Collect a sample of cells from your LeviTube culture and place in a labeled tube.
3) Wash this sample with PBS to remove the media and serum. For multiple samples a bench top quick spin is recommended. When aspirating always use the CubeMagnet to hold the GEMs in place.
4) If the sample is to be fixed, then perform that step now. We have successfully used various percentages of paraformaldehyde to fix cells on the GEM. Use the same percentage of fix and length of fix as you would typically.
5) For targets found within the cell, permeablize the membrane with a mixture of PBS and detergent (e.g. Triton X-100). If necessary for your antibody, blocking solutions may be used to reduce non-specific labeling.
6) Apply the primary antibody and incubate.
7) Wash out the primary antibody and re-block if necessary.
8) Apply the secondary antibody and incubate.
9) Wash out the secondary antibody.

10)Aspirate the wash buffer and apply the mounting medium (see Additional Tips below).
11) Pipette the sample and mounting medium onto a slide. To reduce sample compression insert a spacer between the cover slip and the slide. Another coverslip or a petroleum jelly bead work well.

## Additional Tips

- When adding the mounting agent be sure to add the mount to the sample. Add a minimal amount of mounting agent to ensure your entire sample will fit on the slide. Adding the sample to the mount is difficult and often results in the loss of part or all of the sample.
- A syringe filled with petroleum jelly is a great way to create space between your coverslip and slide. It's easy to make and will prevent sample compression. Melt the petroleum jelly in a water bath set to greater than 38ºC. Pour the melted petroleum jelly into a syringe carefully. Allow it to cool and begin using. If you use a Luer-Lok syringe, then you can change the size of your spacer by changing the needle.
- A post-fix and cure can greatly increase the clarity and life-span of your samples. After washing out your secondary but before mounting, add the fix solution used in Step 4. Fix for 5 to 15 minutes. Wash out the fix and mount as usual. Use either a glycerol mount with DABCO or a commercially available anti-fade product. In either case allow the sample to rest overnight at -20ºC. The sample should be stable for months if not years.