Charles A. Garber, SPI Supplies cgarber@2spi.com

Home-Made Cell Tack

We've used gelled agarose to cover & hold monolayer cells in place when treatment leads to cell lifting. Low-gelling temperature agarose (Type VII) (Sigma A 4018 or A 0701) can be made as a 2% stock solution in "phosphate-buffered saline" (PBS) (boiled then kept at 42° C to remain liquid).

1) Immediately before applying to the cells (with the experimental and/or control treatment), dilute the stock to a final agarose concentration of 0.8% in 2X culture media (37° C) appropriate for the cells.

2) Apply a volume of the agarose solution to the monolayer, incubate at room temperature or 4^o C to gel the agarose.

 Transfer the slide to the incubator to keep the cells metabolically active.

Since the cells are to be visualized on the coverslip, the cells and liquid agarose should be covered with a CoverWell Perfusion or CoverWell Imaging chamber gasket. These devices adhere to the coverslip, creating a very thin culture chamber that would be viewable from the bottom (or top if inverted). CoverWell chamber gaskets are available in different dimension and depths from Molecular Probes (http://www.probes.com). (Search term: Cover-Well).

> Kristen R. Ross and Micheal E. Berens, Barrow Neurological Institute kross@chw.edu or mberens@CHW.EDU

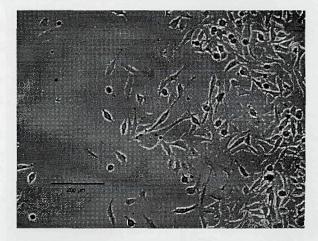


Figure 1. Untreated cells growing in MEM + 10% FBS media.



MICROSCOPY

We appreciate the response to this publication feature - and welcome all contributions. Contributions may be sent to Phil Oshel, our Technical Editor at:

Mr. Phil Oshel	Tel.: (608)833-2885
PO Box 620068	Fax: (608)836-1969
Middleton WI 53562	eMail: oshel@terracom.com

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How Long Should A Gold Sputter-Coater Target Last?

This is not that uncommon of a question. The first rule of thumb is that if it does not look like gold, then it probably is not gold. However, there is one exception and that is when the sputtering process creates some kind of surface structure that leads to an optical effect, there may be a nongold looking gold color (more like gray). But this typically does not adversely effect the sputtering rate.

Other cases would be when either a) contamination from external sources (e.g., finger prints) or b) build up of contaminants from the use of gold with insufficient purity. If a), then the "problem" is solved by a good solvent washing and scrubbing with something like acetone. If b), then it will not rub off with solvent and it would be something building up in the way of impurities from within the gold foil itself.

There is a cost associated with taking gold from 0.98 to 0.99 and even more of a cost to 0.999 purity, then to 0.9999 or 0.99995 there is still more cost. In other words, while high purity gold is desired in the cathodes, because of the build-up of contaminants, the higher purities do cost more money. Putting it another way, a cathode of 0.9999, for example, is a lot more expensive than one that is 0.98 or 0.99, even though the net gold content is about the same.

I won't even begin to speculate on how many would see a difference between 0.99995 vs. 0.9999 or even 0.999. But there is a point where the impurity elements that do not sputter begin to build upon the surface, resulting in a mostly nongold layer. It is my understanding that the original equipment manufacturers of sputter coaters, and also the main firms offering replacement cathodes, supply only cathodes at the higher end of the purity scale because of this accumulation of a non-gold layer on the surface of the target. If gold is obtained from non-traditional EM sources as alternatives, it is important to see the documentation to know that the gold is high purity, but a number of the alternative sources do not deal in such high purity gold.

If lower-purity gold from a non-EM supplier source was used, and there might be a build up of alloying or contaminating elements, try polishing off this layer in a metallographic polishing table in order to renew the original composition that apparently did work for some time. If this is done, I would be most appreciative if the experience is shared with the microscopy community, either on the MSA microscopy listserver, or in *Microscopy Today*, be it good or
