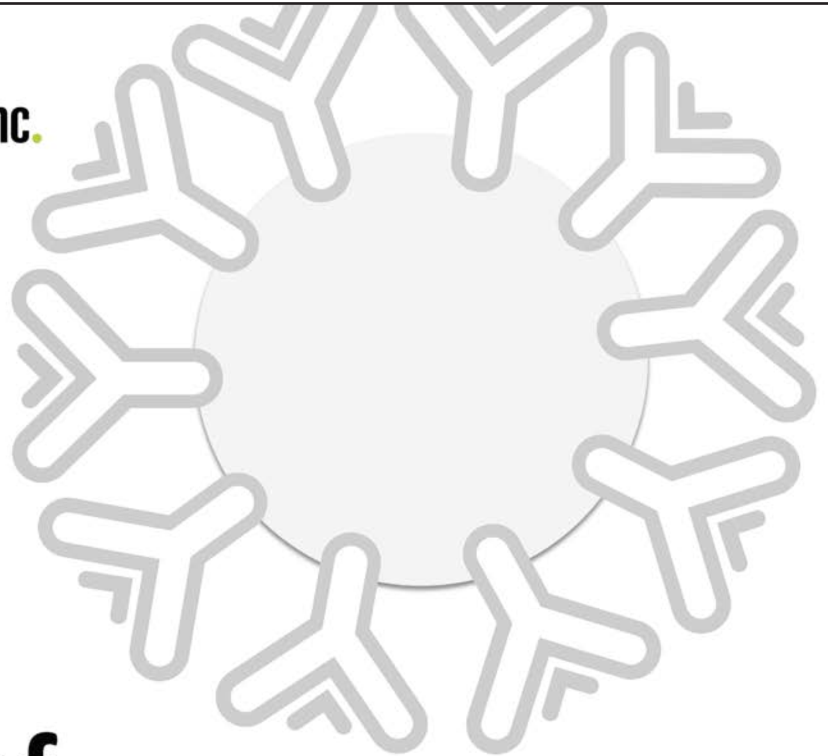


The Art of Microsphere Coating

This is a pdf version of our Microsphere Coating webinar held on 02-12-2015. If you prefer, you can also view the video from our website at www.bangslabs/support/technical-support.

This webinar provides an overview of a general microsphere coating process.

WELCOME!

Kathy Kilbride, *Technical Service Manager*

Sharmi Isaiah, *Materials Manager*

Amy Royal, *Marketing Guru*

WELCOME!

OBJECTIVES

Good protein coverage

(high specific binding, low nonspecific binding)

Specific coating

Blocking

Preserve characteristics during active shelf life

When coating microspheres, the primary objective is often to achieve good coverage.

This is achieved through the specific coating (Ab or other ligand) and blocking.

It is also important to preserve the quality (activity) of the coated microspheres during the intended shelf life.

MICROSPHERES & PROTOCOLS

Microspheres come in all

shapes & sizes

....and so do coating protocols

This and other coupling strategies may be generalized to microspheres of different diameters and matrices (e.g. polymer, silica, magnetic), and may be scaled up or down as appropriate.

Some level of protocol optimization will likely be needed.

PREPARATION

Have the right tools for the task

Pipet

Centrifuge

Bath sonicator

Rotator

Test tubes, etc.

Vortexer

Microscope

BASIC TOOLS FACILITATE MICROSPHERE HANDLING:

Calibrated Pipet to dispense microspheres and reagents

Microcentrifuge for washes / separations of spheres ≥ 0.5µm. Ultracentrifugation devices (e.g. Vivaspin®) or dialysis may be used for spheres <0.5µm. Rare earth magnetic separators are used for superparamagnetic microspheres.

Bath sonicator to prevent or treat aggregation.

Tube rotator for gentle end-over-end mixing (e.g. ~8rpm)

Microcentrifuge tubes, etc. for reactions, washes, etc.

Vortexer for brief mixing. (There must be sufficient volume for vortexing [>500µL] and it should be used with caution due to the potential for loss of some beads / reactants in tube caps, etc.)

The **microscope** is one of the most important tools for working with microsphere suspensions, i.e. to gauge the quality of suspensions (presence of aggregation, etc.) and identify need for treatment such as sonication.

PREPARATION

Coating components

Antibody
Microspheres (COOH)

Crosslinker *(EDAC)*
Blocking molecule (BSA*, casein)
Surfactant
(Tween® 20, Triton-X® 100)
Antimicrobial agent
(NaN_3, ProClin®)*
Buffers:
Coupling (50mM MES pH 5.2)
Wash / Storage
(10mM Tris pH 8.0 + 0.05% BSA + 0.05% ProClin® 300)
as buffer component

COATING COMPONENTS TYPICALLY INCLUDE:

Antibody or other ligand
Microspheres e.g. carboxylated Polystyrene, as used with the PolyLink™ kit (Cat Code PL01N)

COATING REAGENTS AND BUFFERS, e.g. as provided in the PolyLink™ kit:

Chemical crosslinker, e.g. EDAC to activate COOH groups on microspheres so that they will bind with the amine-containing ligand.
Blocking molecule, e.g. BSA*, casein or other protein; synthetic polymer or detergent to block bare patches on microsphere and deter nonspecific binding. *(See our Microsphere Reagent Development booklet for suggestions and MW's)*
Surfactant, e.g. non-ionic surfactant such as Tween®20, Triton-X®-100, typically used at 0.1 – 0.1% w/v in final storage buffer, or very low concentration in wash buffers (0.0005% w/v) to address persistent aggregation
Antimicrobial agent, (e.g. sodium azide, ProClin®) to safeguard against contamination. It is important to understand the treatment spectrum of the specific agent, and whether it is a biostat or biocide.
Buffers for different steps, e.g. general wash buffer such as PBS, coupling buffer (e.g. MES pH 5.2 is suitable for EDAC activation), blocking buffers (e.g. BSA-containing buffer used for blocking and post-coupling washes), storage buffer with appropriate additives to achieve required shelf life (e.g. antimicrobial agent)

POLYLINK KIT COMPONENTS

EDAC IS EXTREMELY HYGROSCOPIC, and will clump and lose activity if exposed to even ambient moisture.

If clumps break apart easily (e.g. when touched with a spatula), this is merely static cling. If clumps are very hard and resist breaking, this is evidence of moisture contamination, and the EDAC should be discarded.

POLYLINK KIT COMPONENTS

Store EDAC desiccated!

EDAC SHOULD BE STORED DESICCATED (i.e. in a desiccator or sealed bag with pillow desiccant) at -20°C.

Desiccators must be properly maintained, e.g. with indicating desiccant in the bottom (changes from blue to pink as moisture is absorbed; it should be changed when ~50% pink), and the edges of the desiccator lip and cover should be prepared with vacuum grease to maintain the seal.

POLYLINK KIT COMPONENTS

Handle EDAC carefully – warm to RT in a desiccator!

EDAC should be warmed to RT in a desicccator to avoid moisture contamination from condensation. It is useful to have a second, i.e. benchtop, desiccator for this purpose.

EDAC should not be stored for lengthy periods once opened. It is a highly economical reagent, and it is often prudent to use a fresh vial / container of EDAC for coating reactions, particularly if the production scale warrants, or it features and expensive antibody, oligo, etc. This is one very important and easy way to mitigate risk.

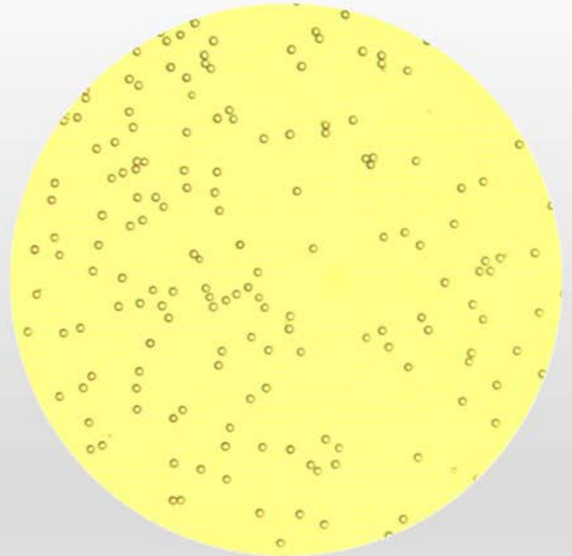
EDAC is sold in small amounts that allow for the use of a fresh vial for each coating. Except for those who are experienced, who are conducting coatings at large scale, and who are able to store it properly, EDAC should not be purchased in large bulk amounts that will be stored for lengthier periods.

LET'S GET STARTED!

First

Roll or rotate suspension for 2hrs prior to sampling / coating

Inspect suspension to ensure that it is monodispersed prior to coating



Bulk bottles of microsphere suspensions should be gently rolled or rotated for ~2-4 hours prior to sampling or coating to ensure the the suspension is monodispersed.

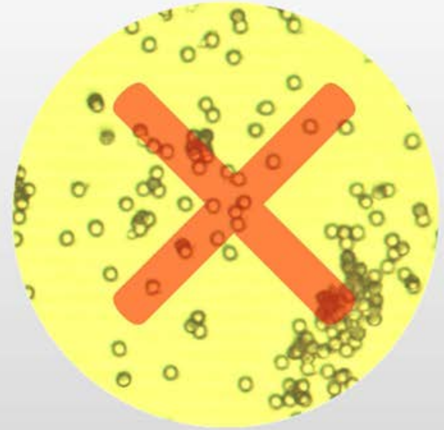
A small amount of suspension should be examined prior to coating to ensure that aggregates are not present. This may be applied to a slide with a transfer pipet, diluted in water or buffer to prevent stacking of particles, and viewed at 400X (40X objective, 10X lens). This magnification will permit the visualization of spheres that are ~1µm or larger, and is also useful for smaller diameters as aggregates would be visible.

LET'S GET STARTED!

If aggregated,

sonicate / vortex / roll / rotate

Inspect suspension to ensure monodispersity



If material is aggregated, it may be possible to return it to a dispersed state with treatment including sonication / vortexing / rolling / rotation and addition of surfactant.

Successive rounds of sonication and rolling may be required. The higher energy of sonication will break apart aggregates, and rolling will distribute surfactant (i.e. the wetting agent) across bead surfaces.

If suspensions have been frozen, aggregation is typically irreversible.

Aggregation that develops over time is often treatable. See TechNote 202, Microsphere Aggregation.

Next

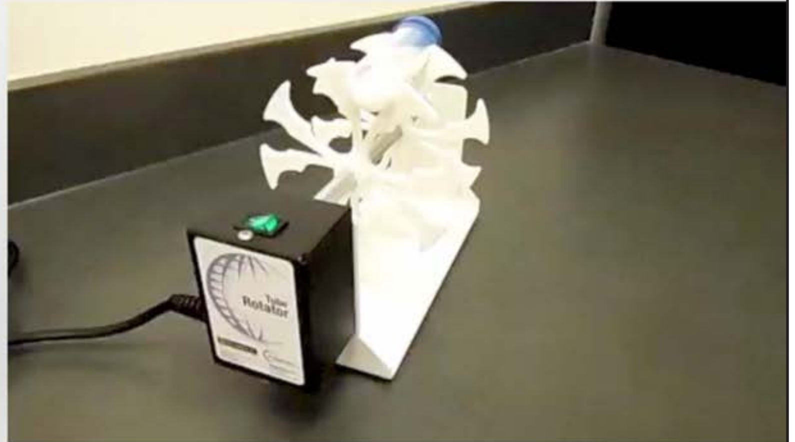
Please see our PDS 644

See Product Data Sheet #644 for the full PolyLink coupling protocol.

NEXT

Briefly bath sonicate the suspension to ensure that suspension remains monodispersed.

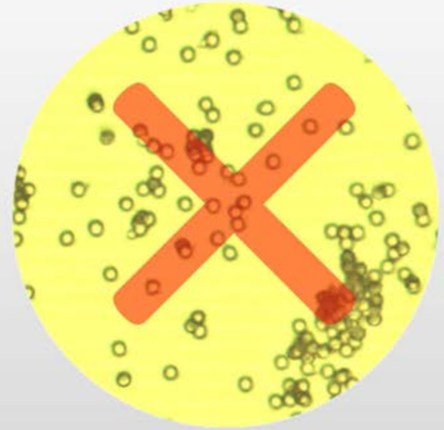
Rotate suspension during protein incubation



The suspension may be briefly bath sonicated after the protein addition (5-10 sec) before being placed on the tube rotator.

NEXT

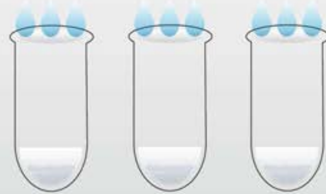
If aggregation has been observed (or as a matter of course), bath sonicate briefly during the RT incubation.



Submicron spheres, or others with a tendency to aggregate during coating, may undergo successive rounds of sonication and rolling during the full protein incubation step, e.g. sonication ~every 15-20 minutes, with replacement on the rotator following sonication. Water bath sonication may be performed for 5-10 sec for small volumes (e.g. less than 1mL), and 15-30 sec for greater volumes.

FINALLY

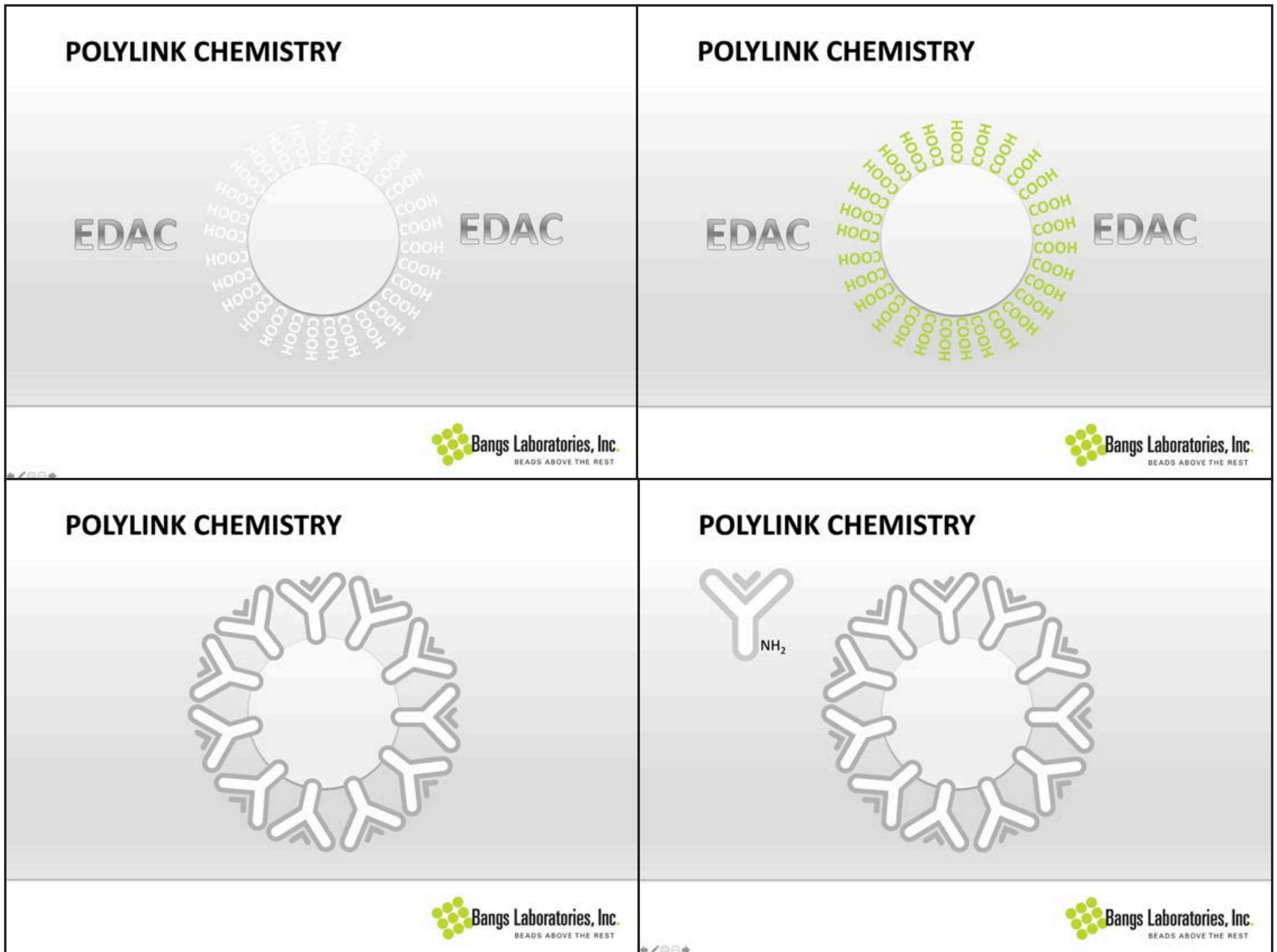
Wash microspheres 2 – 3X



Store in Wash / Storage buffer

At the conclusion of the protein incubation, wash microspheres ~2-3X in Wash/Storage buffer.

As the Wash/Storage buffer in the PolyLink kit contains a blocking molecule (BSA), this also serves as the blocking step.



The basic PolyLink protocol is a general one-step coupling protocol (i.e. no washing after EDAC activation / before protein addition).

This protocol may serve as a starting point for further optimization, and there are also many alternate protocols. See TechNote 205, Covalent Coupling for examples.

IS THAT ALL?

No!

Microsphere optimization

Base bead screening

Antibody screening

Antibody titration

Blocking system

There are many parameters and points of optimization to consider when developing a coating protocol. While the PolyLink protocol is a good general protocol, you may wish to conduct further optimization (and use Design of Experiment principles / software) when developing a microsphere reagent for commercial purposes.

Points of optimization may include:

Base Bead screening: matrix (polymer, silica, magnetic), surface titer, diameter

Antibody screening (cross-reactivity, specific activity, nonspecific binding)

Antibody titration (maximally coated surface, or something less? Fully [ligand] coated surfaces may not be necessary [waste reagent / $], or even lead to increased nonspecific binding in the assay system.

Consideration should be given to the blocking system. Blocking molecules have different characteristics such as MW, charge, hydrophobic / hydrophilic moieties, NSB characteristics.

IS THAT ALL?

No!

Evaluation

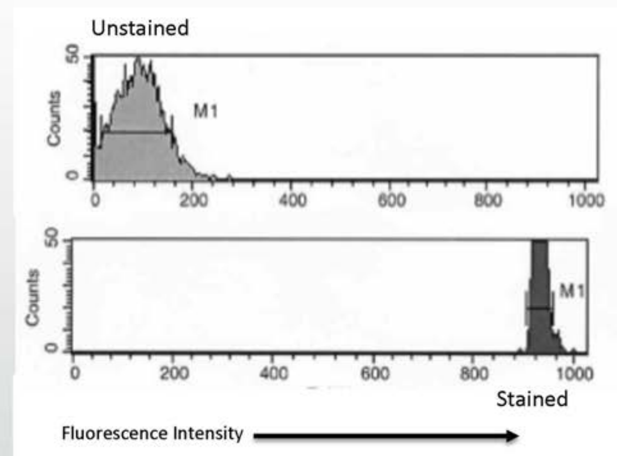
Assess bound protein (TN205)

Assess performance in assay

Assess stability

Need for re-optimization?

(microspheres, other components,
assay parameters)



There are many different ways to assess the quality of a coating through the coating development process (i.e. to determine the need for further optimization), for batches of finished reagent (i.e. for release of product), and to assess stability / shelf life. See TechNote 205 for a number of strategies.

RECAP

- Good general protocols – starting point from which to optimize
- Carefully select and screen components (beads, Abs)
- Design of experiment (DOE) principles / software for optimizing different components and parameters
- Use fresh reagents / take care of stored reagents
- Maintain the quality of the suspension (roll/rotate, do not freeze, do not contaminate)
- Your microscope is your friend

CONCLUSION

And the Secret is:

It's a bit of work…

But fame and fortune await…

Thank you for attending our Microsphere Coating webinar!

Please feel free to contact us if we can be of assistance

www.bangslabs.com 800-387-0672 info@bangslabs.com

THE ART OF MICROSPHERE COATING: AN INTRODUCTION

Question and answer section

Q: We have been having lots of issues with bead loss during washing steps. Especially during activation when no surfactant is present. Do you have any tips?

A: Inclusion of a low level of surfactant in the wash buffer may reduce stickiness. Typically something like 0.01 – 0.0005% of a non-ionic surfactant such as Tween® 20 or Triton®-X 100 can improve handling without a significant compromise in coupling efficiency. If needed, you may also rinse reaction vessels / tubes in a bit of the same surfactant-containing buffer to deter sticking.

If a "smear" is observed on the side of the tube after centrifugation, this is likely due to hydrophobic interactions. In addition to or instead of added surfactant, you may adjust the centrifugation protocol (slightly longer time / greater force) to encourage pelleting. Following separations, you might also examine decanted buffer to find if beads are being lost at certain stages. Adjustment of the centrifugation protocol would also be helpful here.

Submicron microspheres are sometimes lost to inefficiencies with filtration or dialysis. Our Vivaspin® ultracentrifugation devices may provide a more efficient option for small volumes.

Q: How do you quantify your beads?

A: Depending on the specific scenario, we use either a gravimetric method to determine % solids (and from this we can calculate an estimated bead count—see TechNote 206 for the equation) or an analytical method to count spheres. For spheres that are larger than 2µm, we utilize a Coulter Z2 to conduct particle counts. We don't have an in-house instrumental method for conducting counts of smaller-diameter (<2um) particles, however, there are now some instruments that note capability in this region, e.g. Izon's qNano. We have also known customers to utilize a hemocytometer to count beads. This offers a rapid / easy option, though we have found that it's often less accurate / precise than instrumental methods due to sampling or other human error, and small sample size.

Q: What buffer is used for washing carboxylic beads?

A: There are many general / biologic buffers that may be used for wash steps, depending on specific aims. For example, PBS (pH 7.4) or similar may be used for standard washes; you may also select specific buffers to prepare the beads for the next step in the coating process. For example, the Coupling Buffer (also used for bead washes immediately prior to coupling) featured in our PolyLink™ kit is 50mM MES pH 5.2. This buffer is free of additives that may associate with the bead surface or otherwise interfere with the coupling reaction; it is also of a pH that is suitable for the activation of EDAC and binding of protein. Buffer pH may be tailored as needed (or alternate buffers selected) to control the speed of the reaction or to achieve a pH that is most fitting for the ligand.