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**Users Guide for Thermogelling PolyVivo PLGA-PEG-PLGA (AK12/AK24)**

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This guide covers the use of thermogelling PLGA-PEG-PLGA Polyvivo products for generated degradable/injectable thermally sensitive solutions. Each step of usage is discussed in detail below.

### **Setup**

Dry State Handling/Transfer These polymers are short PLGA-PEG-PLGA triblocks and as such are extremely sticky and viscous in their dry and natural state (Fig 1). If you want to subdivide/move to a new container avoid using a spatula as it will stick strongly to anything it touches. A better idea is to make up the aqueous solution in the bottle the thermogel came in (e.g. adding 5ml of saline to ~1g AK12 would yield a 20% solution) and then transfer/subdivide the solution of known concentration. Note that the mass listed on the side of the bottle is the exact mass as determined by analytical balance at the time the material was placed into the tared bottle. You can use this accurate mass for your mathematical calculations. If you do need to subdivide the solid, the best way is to gently warm the dry gel to ~37C for a brief period and then it softens to where it can be poured from one container to another. This does not mean the material is not a thermogel, it displays thermogelation in water, in dry state its behavior is similar to any viscoelastic material. When not in use storage at -20C is recommended.



Figure 1. Vial containing ~1g PolyVivo AK12 in dry state. Appears like a semi-viscous gel.

Dissolution Thermogels dissolve best cold. This means that (counter-intuitively) the best way to dissolve it is to put it in a refrigerator (4-10C) (whatever you do, do not heat to dissolve! This will only damage the polymer and make it dissolve even slower). The dissolution also takes some time (at the very least overnight) so best to let it dissolve overnight or even better a few days in advance and then use it. **DO NOT WAIT UNTIL “READY TO USE” TO START DISSOLUTION, THERE IS NO MEANS TO DISSOLVE THE MATERIAL IN WATER IN ANY SHORT TIME FRAME.** Keep this in mind when planning timing for your animal surgeries, you will need to dissolve the polymer well in advance of your surgery date. Stirring and vortexing can improve dissolution

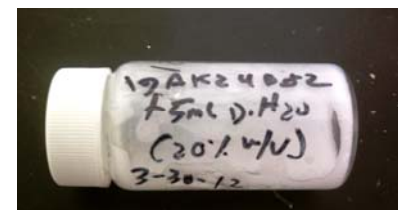


Figure 2. dissolved solution of 20% AK24 has whitish, “soapy” appearance.

rate though sonication has limited benefit due to increased heating. At 4C the polymer solution is stable for about 2-3 weeks. After a month or so in aqueous solution in the fridge it will gelate due to degradation and not work as a cold-water soluble material so if you find some "left-overs" much later on chances are they may not be good. When dissolved the solution will be whitish with a tendency to form foam when shaken due to micelle structure formed (Fig 2).

### Mixing with deliverable

The best means of incorporating the deliverable is to dissolve it directly into the cold aqueous solution of the thermogel as it is. Due to the micelle-type structure of the thermogel polymer it does have some capacity to improve hydrophobic drug solubility as well as aid dispersion of microparticles. Mixing is best accomplished while the solution is kept cold for optimal polymer solubility.

### Testing

### *In Vivo Implantation/Injection*

Make sure to keep the polymer solution cold right up to the time of surgery. The best method is to take a small styrofoam box in with you to surgery with the polymer solution in it and crushed ice. These thermogels can be a little "trigger-happy" when it comes to gelling and will start to solidify up even if you are working in a warm room (25-30C) so for injection/insertion purpose keeping it cold is optimal. Inject the solution quickly into anesthetized animal (use animal model in accordance with your local IACUC) with a syringe/needle of appropriate size for animal and desired site. Gelation should occur rapidly upon the warming of the solution inside the living animal. Note gelation can also occur with the heat of your hands/heating in the needle so a rapid injection is best.



Figure 3. Animal preparation anesthesia (in this case by isoflurane) and shaving of injection region.\*



Figure 4. Syringes of thermogel cooled on ice prior to injection



Figure 5. Injection (in this case SubQ dorsal region) should be rapid with minimal syringe handling.

\* - images taken from a procedure using a generic thermogel for injection, not necessarily an Akina product

Treatment and handling of the animal post injection is dependent on your deliverable and protocol. Material will form a gel at injection site which will remain until degradation/re-dissolution occur. Substantial cooling of the location or animal is unadvised (e.g. application of ice-packs to the injection site) as it may redissolve the gel.

### *In Vitro Drug-Delivery Tests*

The material can also be utilized for in-vitro tests. The best means to do this is to place a known amount of the gelling solution mixed with your deliverable into the bottom of a test vial and heat to 37C for a short time (10min) to gel. Afterwards add your desired volume of release media (for a drug delivery test) and incubate at 37C. Refresh/Test the release media for drug content at desired time-points relevant for your test time period. Figure 6 shows an example of putting 0.5ml of thermogel in the bottom of a tube followed by 1 ml of test media. This is one means of testing in vitro but there are several others. Note: material's gelling is not so strong as to form a solid upon introduction to excess hot water.

### *Alternate Dissolution Method*

An alternate dissolution method has been tested at PolySciTech utilizing DCM to pre-dissolve the material into the water. In this method AK24 was first dissolved in dichloromethane at ~10-20% w/v. This solution was subsequently added drop-wise to rapidly stirring water (2000RPM via overhead screw-type paddle, Fig 7). The solution was stirred open to air at room temperature for 1 hour afterwards the vial was capped and placed in a refrigerator overnight (equivalent to 10% w/v AK24/water assuming all DCM evaporated during stirring). This method was noted to generate a more even solution of the polymer in the water more rapidly than refrigerator dissolution alone of the solid polymer. Despite this the ultimate fate of the DCM is unknown and some may still be retained in the solution. Post dissolution the 10% w/v AK24 solution was tested and found to also possess thermogelation properties.

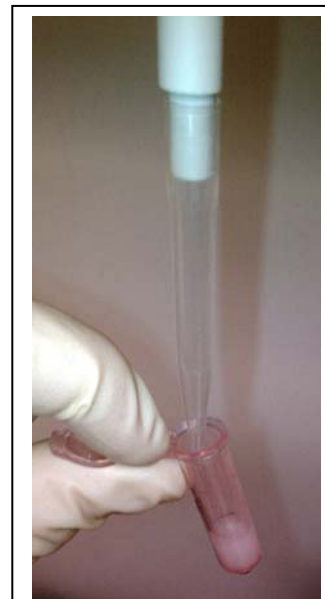


Figure 6. Adding gel into test vial.

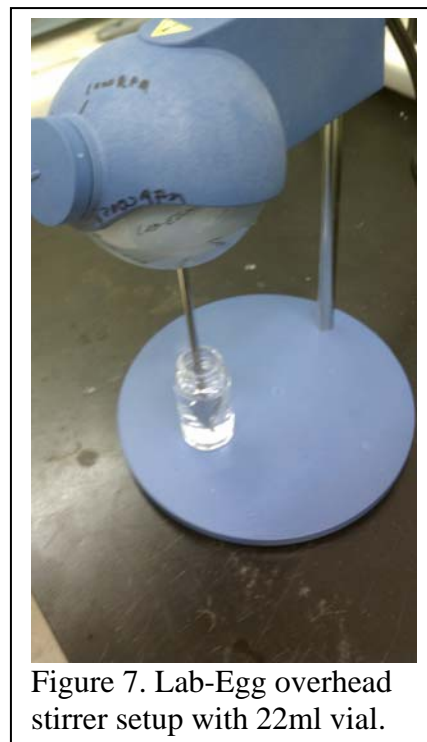


Figure 7. Lab-Egg overhead stirrer setup with 22ml vial.