

TITLE: Protocol for Preparing Tween Solutions

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1 Overview

The goal of this document is to describe a procedure for mixing Tween solutions to facilitate the suspension of hydrophobic particles in water. Applications include, but are not limited to, suspending particles for use as density marker beads in Percoll or other gradients, or for use in flow visualization. This document will outline a procedure for making approximately 100ml of 0.1% Tween by mass. Adjustments to the ingredients and their quantities can be made depending on the need and the application; these adjustments and other considerations will be discussed in §5.

2 Materials to be Used

The following is a list of the materials necessary to create 100ml of solution as used for Cospheric DMB products. For varied applications different concentrations, quantities, or surfactants may be appropriate; see §5.

- 1) Clean jar for mixing and long term storage of final solution (size based on need)
- 2) Tween20 biocompatible surfactant (other surfactants can be substituted)
- 3) De-ionized water
- 4) Scales: accurate to 0.01g with at least 500g capacity (capacity based on need)
- 5) Output control hotplate suitable for boiling water
- 6) Medium sized pot (size based on need)
- 7) Thermometer: accurate to at least 1°C
- 8) Pipette or syringe x2 (size based on need)
- 9) Tap water
- 10) Insulated gloves
- 11) (optional) Burette stand and clamps

3 Preparing Tween Solutions

PREPARE A HOT WATER BATH

- Begin by filling the pot about 80% full of tap water, this is to allow room to submerge a jar in the water.
- Place the pot on the hotplate, insert the thermometer into the water such that the thermometer does not contact the walls of the pot (a burette stand and clamps may be useful for holding the thermometer in the water).
- Turn the hotplate on to high. Keep an eye on the thermometer, a stable temperature of approximately 80°C is desired. When 80°C is reached, the hotplate can be turned down to maintain the temperature.
- While the water is heating, proceed to prepare the Tween mixture.

PREPARE THE TWEEN MIXTURE

- Place the jar on the scales and tare the scales.
- Using a pipette or syringe dispense 0.10 grams of Tween20 into the jar.

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- Fill the jar with de-ionized water until the scales read 100.00g (i.e. add 99.90g of water). This can be done efficiently by pouring directly from the vessel holding the de-ionized water into the jar until the desired weight is approached but not met. The final amount of water can be carefully dispensed with a pipette or syringe to achieve the desired weight accurately.
- Secure the lid on the jar, it is now ready to be mixed.

MIX THE SOLUTION

- When the hot water bath has stabilized at approximately 80°C, begin heating and mixing the Tween solution.
- While holding the jar firmly by the lid (ensure the use of insulated gloves, long sleeves and safety glasses), gently submerge the bottom of the jar into the water bath attempting to match the two water lines. Be careful to not allow the water in the pot to overflow. Do not rest the jar on the bottom of the pot as it can cause uneven heating and stress on the jar.
- Hold jar partially submerged for 20-30 seconds.
- Remove jar and swing it in a gentle circular motion for 20-30 seconds. Be careful as condensation can make the jar slippery. To prevent risk of injury, do not swing the jar quickly or vigorously or hold the jar high above a table or the floor.
- Repeat the submersion and swinging process several times until the jar is quite warm to the touch (approximately 5-10 minutes).
- When finished, the solution should look uniform. If the Tween is not mixed properly, "strands" of viscous Tween may be seen swirling and floating in the water (this can be very difficult to see at this low concentration).
- Once mixing is complete, leave the solution to cool overnight before using.

4 Using Tween Solutions to Suspend Particles

The specifics of this section will pertain to creating Cospheric's DMB products which are defined to have 20% solids in 2.5ml. The process is easily modified to other situations.

- Using a 1 dram vial, add 0.5g of the desired microspheres. (Any container and hydrophobic particles may be used).
- Using a pipette or syringe, dispense 2.0ml of the Tween solution made in §3 on top of the spheres. (make sure you add the Tween to the spheres, not the spheres to the Tween).
- Secure the cap on the vial and gently invert the vial several times to mix without creating soap bubbles.
- Make sure that the vial is labeled appropriately or placed in a re-sealable bag labeled with products code, lot number, concentration, and quantity.
- The final product should be left to sit 1 to 2 days before shipment or use to ensure complete wetting of the spheres.
- Once the product is fully wetted, the solution can be diluted down to any concentration.

5 Considerations for Varied Applications

This protocol has been specific to creating a large amount of solution suitable for creating many samples of Cospheric's DMB product. Consider the needs of the application, if only a

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few samples need to be prepared, then only a small amount of solution needs to be prepared; likewise if a large quantity is needed, it can be made in a single large batch. When making these adjustments, one should not only change the amount of surfactant and de-ionized water, but should also use appropriate containers, pots, scales, dispensation equipment, and heating equipment.

If there is concern that the concentration of Tween in the solution may affect an experiment unpredictably, a lower concentration can be used. Successful wetting has been observed with as little as 0.01% Tween by weight. When reducing the concentration, more time should be allowed for the Tween to mix and for particles to be wetted, be aware that at very low concentrations it may take up to a week for particles to become completely wetted. Conversely, if the effects of Tween are not a concern but the time investment is problematic, wait times can be reduced by increasing concentration.

Tween20 has been used in this protocol specifically because it is biocompatible which makes it suitable for many density marker applications, Tween80 can also be used to the same effect. If biocompatibility is not an issue, then any concentrated surfactant can be used. It is recommended that one begins with a concentration of 0.1% surfactant by weight; this strikes a balance between having enough surfactant to wet the particles well and being a low enough concentration to have negligible effect on most experiments.