

SOP: R001

Preparation of 32% Triton X-114

Materials and Reagents:

1. 1 Liter bottle
2. PBS (Phosphate Buffered Saline) 1X, pH 7.4 (Gibco cat# 10010-023)
3. Triton® X-114 (Sigma cat# X114-1L)
4. Stir Bar
5. Stir Plate
6. 4°C refrigerator
7. 37°C Water Bath
8. Serological Pipets
9. Pipetaid

Protocol:

1. ____ In a 1 liter bottle combine 150 ml of Triton® X-114 and 150 ml of PBS.
2. ____ Place on stir plate and mix thoroughly. Several hours is recommended.
3. ____ Transfer mixture to 4°C and let stand until the mixture is clear (note 1).
4. ____ Transfer the mixture to a 37°C water bath and incubate until a biphasic occurs (note 2).
5. ____ Carefully remove the top layer of the biphasic and discard (note 3).
6. ____ Add an equal volume of PBS to the Triton® layer that was not removed (note 4).
7. ____ Return to the stir plate at room temperature and mix until the solution is clear.
8. ____ Return mixture to the 37°C water bath and incubate until the second biphasic occurs (note 5).
9. ____ Repeat steps 5-8 once more for a total of 3 biphasics.
10. ____ On the third time, remove as much of the top layer as possible, then transfer the remaining Triton® to a stir plate to recombine any liquid not removed (note 6).
11. ____ Store at 4°C until use.

Notes:

1. Overnight incubation is recommended. If the mixture is not mixed well enough it will never go completely clear. If this happens remix and incubate again.
2. If the biphasic does not occur after several hours of incubation, continue to step 6 (which would be to double the volume with PBS). The biphasic may be too little to see; therefore, the liquid would not be able to be pulled off regardless.
3. Be carefully to not disrupt the Triton® layer.
4. For example: from 300 ml of starting material, after the 1st biphasic 50 ml is removed from the top layer, then 250 ml of PBS will be added back to the Triton® layer.
5. A biphasic will definitely occur from this step forward so do not proceed until a biphasic occurs. If a biphasic does not form, incubation can be carried out at 56°C. The volume of the biphasic layer will greatly increase on the 2nd and 3rd pass.
6. This will yield about 400-450 ml of 32% Triton® X-114.