

## Sperm Activation

3 methods are available to activate sperm depending on the degree of embryonic synchrony and the amount of embryos necessary for the experiment.

1. Activation by exposure to chorionated eggs: add 25  $\mu\text{L}$  of concentrated sperm to 5 mL SW containing 100  $\mu\text{L}$  chorionated eggs; shake gently (20 rpm) for 15–60 min.

Let chorionated eggs sediment to the bottom of the tube; the activated sperm suspension can be kept at 4°C and used to fertilize for several hours.

2. Activation by alkaline SW (with NaOH): dilute 10  $\mu\text{L}$  of concentrated sperm in 2 mL of SW and then add 4–12  $\mu\text{L}$  of 1 N NaOH. Wait 1 min and add the activated sperm to eggs.

You have to find the right concentration of NaOH (usually 8  $\mu\text{L}$  work well). This activation works very well with *Phallusia* sperm but should be done each time, just before fertilization.

3. Activation by alkaline SW (with Tris–HCl buffer): Add 3–6  $\mu\text{L}$  "dry" sperm to 500  $\mu\text{L}$  pH 9.5 sea water and agitate for 20 min (change the pH with NaOH, not KOH which is spermicidal).

25  $\mu\text{L}$  of 1 M Tris–HCl buffer (pH 9.5) to 0.5 mL of SW. To this alkaline SW, add 2.5  $\mu\text{L}$  of concentrated sperm; mix and wait 1 min before fertilizing. The sperm suspension can then be used to fertilize for 1 hour.