Standard Operating Procedure

Title: Preparation of fixative solution.

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Background

The most common usage of fixatives in the laboratory is for the preparation of tissue for histological examination. Fixatives, particularly the formaldehydes, work by cross-linking proteins within the cells of tissues thereby preserving the structural integrity of the cells after the death of the animal. These chemicals can fix the tissues of the user just as readily as experimental tissue, and are therefore highly toxic and carcinogenic with prolonged exposure. The ideal situation is zero exposure to these chemicals.

Body parts most at risk

Cornea of the eyes, tissue lining the respiratory tract, skin.

Equipment

Scales
Weighboats (or vessel to contain the fixative)
Fixative (eg paraformaldehyde)
0.1M Phosphate buffered saline (PBS)
2L Beaker
1L laboratory bottle
Stirring hotplate
Magnetic stirrer
Sodium hydroxide pellets
Glass funnel
Retort stand

Personal protective equipment required

Safety goggles (or even better a full face shield)
Gloves
Lab gown

Procedure

- Place 600mL of PBS into the beaker and heat to not more that 60°C
- Add 10 or so pellets of sodium hydroxide (paraformaldehyde requires OH^- ions in order to depolymerise and dissolve).
- In a fume hood weigh out 40g of paraformaldehyde and add to the beaker.

  Risk: At no time should there be a clear path between the paraformaldehyde solution and the eyes of the user. **ALWAYS** wear safety glasses when using fixatives.

Procedure (cont.)

- When dissolved (solution should be completely clear), allow to cool to at least 40°C and filter into laboratory bottle, make up to 1L with PBS.

  Risk: Bottles and beakers can get heavy when full. It is sometimes awkward pouring solutions into filtering apparatus when fume hood sash is at appropriate operating height. Raise sash enough to allow comfortable hold on glassware for this step.
- Adjust pH of solution to ~7.2-7.4 with concentrated hydrochloric acid (HCl) solution.

  Risk: HCl is a gas thus the concentrated solution used at this step gives off fumes. Fumes will dissolve in the moisture of your respiratory tract if inhaled and form HCl solution, irritating respiratory tract. Perform this step in a fume hood.
- Cool to 4°C before use.
- Should make up as close as possible to the day needed. Avoid using paraformaldehyde more than a week old, as fixation efficacy may be reduced.

Disposal of waste

Any waste powder should be disposed of in yellow biohazard bags. Take care to contain powder to reduce accidental exposure when handling disposal of bags.

DO NOT pour liquid waste down the sink. Place excess waste into 20L waste container, reuse container until full then dispose as chemical waste.

References

1. Sigma (2000) Paraformaldehyde MSDS
2. Leong, A S-Y. Fixation and fixatives. http://home.primus.com.au/royellis/fix.htm (accessed 25/5/07 Fri)