School of Engineering

DIVISION / UNIT: Trace Metals in Medicine Date: 10-Nov-2016

PRINCIPAL INVESTIGATOR: James Everett

SUPERVISOR/MANAGER: Dr Joanna Collingwood

TITLE OF PROCEDURE OR ACTIVITY: Use of Sodium Phosphotungstate for Negative Staining

Location of activity: F013

Laboratory work: Yes

1. SUMMARISE THE ACTIVITY

i) Overview of work: Sodium phosphotungstate is used to increase the contrast of structures (in this case protein aggregates) for examination under electron microscopy

ii) Description of procedures:

1. Prepare a 2% aqueous solution of the sodium phosphotungstate in distilled water and adjust the pH to 7.0 with 1M sodium hydroxide.

2. Mix equal quantities of protein sample and sodium phosphotungstate (approximately 10 µl of each).

3. Place a 10 µl drop of this mixture onto a formvar/carbon TEM grid held by tweezers. Leave for ~20 sec and then remove the solution by wicking with filter paper.

4. Air dry and store in grid box. A wash with distilled water after drying may be necessary if crystalline products are present on the TEM grid.

iii) Substances used:

Distilled water

Sodium phosphotungstate powder

Sodium hydroxide solution

iv) Quantities used and frequency of use:

Stock Solution: 2 mL of 2% sodium phosphotungstate stock (40 mg sodium phosphotungstate in distilled water; adjusted to pH 7 with 1M sodium hydroxide). Stock stored within sealed container in cool, well-ventilated place.

Typical working quantities: 10-50 µl of 2% sodium phosphotungstate stock (depending on sample number) used at a given time.

Variable frequency of use: making the 2% stock solution will be a rare occurrence, typically twice per year. Use of the stock solution for staining of grids will take place during short periods of frequent use (e.g. daily use for 3-4 days), followed by prolonged periods (e.g. several months) without use.

2. IDENTIFY THE HAZARDS AND ASSESS THE RISKS TO HEALTH AND SAFETY

Identify hazards:

Sodium phosphotungstate:

H302 Harmful if swallowed

H315 Causes skin irritation

H319 Causes serious eye irritation

H335 May cause respiratory irritation

Precautionary statement(s):

P261 Avoid breathing dust.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove

contact lenses, if present and easy to do. Continue rinsing

Sodium Hydroxide:

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage

Precautionary statement(s):

P280 Wear protective gloves/ protective clothing/ eye protection/ face

protection.

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.

P305 + P351 + P338IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Information from Sigma Aldrich SDS sheets.

i) Identify <u>potential</u> route(s) of entry into the body:

Inhalation Yes / No Yes	Ingestion Yes / No Yes	Splash in eyes or mouth Yes / No Yes

ii) Identify any particular groups of workers who may be at increased risk:

None

iii) Could a less hazardous substance (or form of the substance) be used instead?

No

3. DECIDE WHAT PRECAUTIONS ARE NECESSARY TO PREVENT OR CONTROL THE RISKS

Specify what measures are required to control the risk:

When creating 2% sodium phosphotungstate stocks, keep volume as low as reasonably possible. Stock solution has a long shelf life, so handling the powder to make the stock solution only needs to be done once or twice per year.

Conduct all work involving sodium phosphotungstate within a fume hood, including weighing out of the powder, and staining of grids. No toxic gases should be produced, so a fume hood with a particulate filter should be used. The hood in F013a is equipped for this purpose.

Wear PPE at all times (see below).

Perform dilution of NaOH from 10M to 1M in the fume hood also, to establish a containment area in the event of a spillage, working with minimum quantities, wearing PPE at all times. Ensure strong acids and bases are kept completely separate.

i) Engineering control measures:

Fume hood equipped with filter for powder handling

ii) Personal protective equipment (PPE):

Lab coat Yes / No Yes	Gloves Yes / No Yes	Eye or face (specify if yes) Yes / No Goggles

iii) Other measures:

a) Containment level

b) Additional control measures

None

iv) Waste disposal procedures:

Dissolve/mix waste sodium phosphotungstate in a combustible solvent for destruction in a chemical incinerator. It is a non-chlorinated substance which is soluble in water, so ethanol and a non-chlorinated waste solvent bottle can be used for this purpose.

For the weigh boats required for powder when stock solution is prepared, rinse with ethanol, dispose of the ethanol wash into the waste non-chlorinated solvent bottle, then dispose of the weigh boat in clinical (yellow) waste bags.

v) Emergency procedures:

In case of fire: Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide. Oxides of phosphorus, Sodium oxides, Tungsten oxide may arise. Advice for firefighters: Wear self-contained breathing apparatus for firefighting if necessary

4. ENSURE CONTROL MEASURES ARE USED AND MAINTAINED

Specify what, if any, checks on control measures are required and state the frequency of inspection needed:

Ensure fan on fume hood is functioning prior to use.

Have a fresh sheet of absorbent ‘bench guard’ on the working area inside the fume hood in case of any spillage.

5. MONITOR EXPOSURE OF WORKERS (IF NECESSARY)

Specify if environmental or personal monitoring is required:

No

Specify if health surveillance is required:

No

6. PROVIDE INFORMATION, INSTRUCTION AND TRAINING TO PERSONS AT WORK

Specify training requirements:

Laboratory good practice and chemical Moodle course (online)

7. SIGNATURES

Name of Assessor: James Everett

Signature: James Everett Date: 10.11.2016

Name of Reviewer: Joanna Collingwood

Signature:  Date: 10.11.2016

RECORD OF PERSONNEL INVOVED

Name	Position and Qualifications	Experience	Start Date	Finish Date
James Everett	PDRA	6 years lab experience	June 2016	Jan 2017
Jake Brooks	PhD Student	1 year lab experience	Oct 2016	Oct 2019

Reviewed 11/05/2021: personnel listed above continue as posts have been extended, both are now PDRAs. Newer members of the laboratory will need to become familiar with the content and formally added to this approval by Prof. Collingwood before performing the procedure.

11th May 2021