

Von Kossa Calcium Stain Kit

Purpose and principle.

Abnormal deposits of calcium may be found in any area of the body. This method depends on treating sections with silver reagent which carries out a substitution reaction with the calcium phosphate in bone forming silver phosphate, this is then reduced to black metallic silver by the action of light.

Kit Contents.

Silver stain stock 250ml Sodium thiosulphate 250ml Van Giesons stain 250ml

Materials required but not provided.

Fixation in 10% formalin, dehydration alcohols, xylene, DPX mountant

Control tissue.

Tissue containing known positive calcium deposits or undecalcified bone.

Procedure

- 1 - Dewax sections and hydrate to deionised water.
- 2 - Place the slide in a small clean staining jar (coplin or smaller to reduce wastage of reagent (plastic staining jars are included) and add silver stain reagent until the tissue is just covered. Either place the staining jar in **bright sunlight** on a window sill or in front of a 60 watt lamp (50-75mm away) with aluminium foil behind the staining jar to reflect the light. Leave for 1 hour or until calcium turns black (checked macroscopically). If UV light is available the time can be reduced to about 10 minutes (beware certain UV wavelengths are dangerous, check equipment with Safety Officer before use).
- 3 - Rinse in 3 changes of deionised water.
- 4 - Place slide in Sodium thiosulphate reagent for 5 minutes.
- 5 - Wash in deionised water for 1 minute.
- 6 - Stain in Van Giesons reagent for 3-4 minutes.
- 7 - Wash in tap water.
- 8 - Dehydrate, clear and coverslip with DPX.

Results.

Calcium salts	black
Osteoid seams	red

Observe and follow the usual good laboratory practise and safety precautions when handling these reagents, use appropriate safety equipment, gloves, goggles, lab coat etc.

Store kit components in the dark at room temperature.

Silver stain reagent will irritate eyes and skin and cause black burns to skin. If in contact with eyes and skin wash immediately in soap and water for skin or use an eye wash for the eyes. If ingested may cause violent gastrointestinal discomfort. Possible carcinogen: equivocal to mutagenic agent.

Sodium thiosulphate may cause GI distress and cause irritation to eyes. If in contact with eyes wash immediately with plenty of water.

Van Giesons stain contains Picric Acid which is TOXIC by ingestion, in contact with skin and Toxic to the aquatic environment. It is also an Irritant and may cause burns to eyes and skin. If in contact with eyes rinse with water bath for at least 5 minutes. If soreness persists seek medical attention. If in contact with skin wash well with soap and water. If ingested seek IMMEDIATE medical advice on what action to take and what treatment to administer.

Spillage & Waste Disposal

In all cases of spillage of reagents above – mop up with cloth or paper and rinse cloths under tapwater to Public Sewer diluting greatly with tap water.
Recycle cleaned packaging as polyethylene.

GCC diagnostics guarantees that the highest quality reagents are supplied with this product to give reliable results time and again and that this product conforms to the information contained in this leaflet. The user should however, determine the suitability of this product for their particular use.

If you wish to report any findings to us or if you require any help or further information please contact us.

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