

CLIN-TECH LIMITED

TOLLEN'S REAGENT

IVD

Product Code 62068



INTENDED USE

The Clin-Tech Tollen's Reagent kit is designed for detecting aldehydes. It produces a 'silver mirror' in the presence of aldehydes, but not in the presence of ketones.

Silver mirror reactions are used in the Fontana-Masson argentaffin reaction test, wherein the reducing groups (such as aldehydes) in cells are stained by metallic silver deposits.

KIT PRESENTATION

<input type="checkbox"/> Reagent 'A'	10 mL
- 10 % Silver nitrate	
<input type="checkbox"/> Reagent 'B'	90 mL
- 0.694 % Ammonium Hydroxide	
- 0.083 M Sodium Hydroxide	

Do not use beyond the expiry date printed on the reagent label.

Concentrations when reconstituted 1 part 'A' to 9 parts 'B':

1 %	Silver Nitrate
0.625 %	Ammonium Hydroxide
0.075 M	Sodium Hydroxide

PROTOCOL: SILVER MIRROR TEST

Preparation

- Mix together 1 part Reagent 'A' and 9 parts Reagent 'B'. Alternatively, add all of Reagent 'A' to the Reagent 'B' bottle.
- Mix by inverting 3-4 times.

Method

- Add a couple of drops of test solution (or crystals) to approximately 5mL reconstituted Tollen's Reagent in a glass test-tube.
- Mix and allow to stand for a few minutes.

PROTOCOL: ARGENTAFFIN TEST

Also required:

0.2 % Gold Chloride solution	64121X
5% Sodium Thiosulphate solution	64131X
Nuclear Fast Red	64242X

Preparation

- Reconstitute Tollen's Reagent as above. Dilute 1 part reconstituted Tollen's Reagent with 3 parts purified water.
- Deparaffinise slides and hydrate.

Method

- Warm diluted Tollen's Reagent to 60-70 °C. Immerse slides in warm diluted Tollen's Reagent for 5 min. Alternatively, immerse slides in unheated diluted Tollen's Reagent for 2 hours. Check slides under a microscope for silver impregnation.
- Rinse slides in purified water.
- Tone sections in gold chloride for 5-10 sec.
- Rinse in purified water.
- Immerse in sodium thiosulphate solution for 1 min.
- Rinse in purified water.
- Counterstain in Nuclear Fast Red (Kernechtrot) for 5 min.
- Rinse in purified water, dehydrate in ethanol and mount in a synthetic medium.

RESULTS

A positive result for aldehydes gives metallic silver (or sometimes a black precipitate), which deposits on the sides of the glass test-tube and surface of the solution, resembling a silver mirror. Ketones require a stronger oxidising agent and therefore do not give this result (except in the case of alpha-hydroxy ketones).

Reducing groups in cells are stained black by the silver, as is melanin. Argentaffin granules are seen in carcinoid tumours. Non-tumour cells which secrete serotonin (i.e. GI-tract epithelial cells) also take up the stain.

Argentaffin granules	black
Serotonin-producing cells / Melanin	black
Nuclei	pink-red
Cytoplasm	pale pink

STORAGE AND STABILITY

Once reconstituted, Tollen's reagent is only stable for a matter of hours (up to 12 hours) and should therefore be made up on the day of use.

TOLLEN'S REAGENT MAY DEGRADE TO FORM POTENTIALLY EXPLOSIVE SILVER NITRIDE. EXCESS SOLUTION AND WASTE (INCLUDING SAMPLES) SHOULD BE DENATURED WITH TWO – FOUR VOLUMES OF DILUTE ACID OR SATURATED SODIUM CHLORIDE SOLUTION, THEN DISCARDED IN LINE WITH LOCAL REGULATIONS REGARDING HEAVY METAL DISPOSAL.

Bancroft, J.D. and Gamble, M. *Theory and Practice of Histological Techniques* (2008) 6th ed. pp 293-4.

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