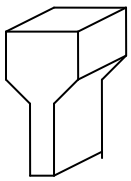
Incubations, alternative for hot-plate

The temperature during incubations is very important. In order to optimise the required temperature(s), a water bath provides a cost saving, easy to control, easy to use and simple solution

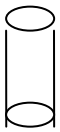
Preparation of pepsin solutions

Pepsin stock solution	Dissolve Pepsin powder with 4 ml deionised water Aliquot in 40 portions of 100 µl and store at -20 °C	 4	 ml	Store at -20 °C
Pepsin diluent - Paraffin sections 0.1 M HCl	Dilute the Pepsin diluent (1M HCl) with deionised water - pepsin diluent deionised water	 5 45	 ml ml	Pre-warm in water bath
- Cytological specimen 0.01 M HCl	- pepsin diluent deionised water	0.5 49.5	ml ml	
- Frozen sections 0.01 M HCl	- pepsin diluent deionised water	0.5 49.5	ml ml	
Proteolytic work solution - Paraffin sections - Cytological specimen - Frozen sections - Paraffin sections - Cytological specimen - Frozen sectionsl	Thaw pepsin stock solution <u>Single slide staining (1-3 slides)</u> - 0.1 M HCl pepsin stock solution - 0.01 M HCl pepsin stock solution - 0.01 M HCl pepsin stock solution <u>Batch staining (5 slides)</u> - 0.1 M HCl pepsin stock solution - 0.01 M HCl pepsin stock solution - 0.01 M HCl pepsin stock solution Mix well and fill staining jar and pre-heat to 37°C	 10 100 100 4 100 2 50 500 100 4 100 2	 ml µl ml µl ml µl ml µl ml µl ml µl	Prepare fresh solution each time

Pepsin digestion:



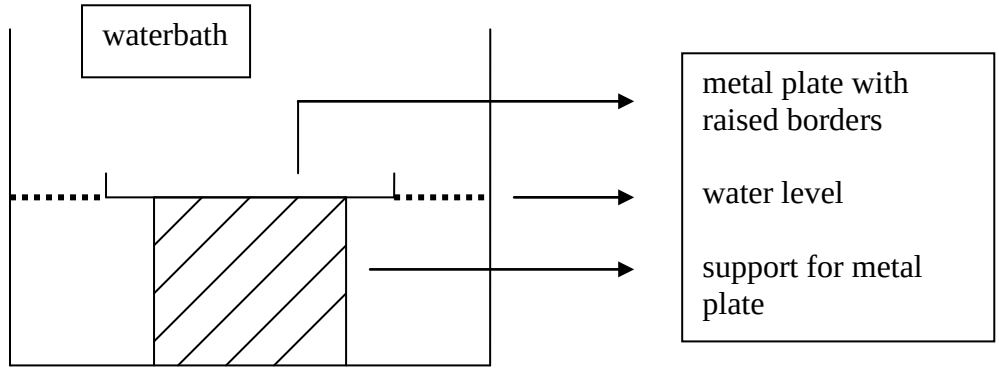
staining jar with lid for 5 slides



staining jar with lid, for 1-3 slides

Prepare the appropriate pepsin <u>diluent</u> (concentration and amount needed), pre-warm in staining jar in water bath set at 37 °C. Check temperature, add pepsin <u>stock solution</u> just before use, mix, add slides and incubate for the required time (in the jar, in the water bath).
- The pepsin <u>diluent</u> can be prepared and pre-heated at the start of the procedure making sure that the required temperature is reached when you arrive at the point of digestion; at that point, check the temperature, add and mix the pepsin <u>stock solution</u> (just before use); add slides and incubate for the required time.
- In case the level of proteolytic work solution is too low (does not cover the slides completely), add something volume increasing to the staining jar i.e. an empty glass tube.
- Do not incubate > 5 slides simultaneously, otherwise the temperature may drop.

Denaturation:



Place metal plate in water bath (prescribed °C, check temperature), add probe reagent and cover slip, place slides on metal plate and incubate for the prescribed time.
- Do not incubate > 5 slides simultaneously, otherwise the temperature may drop.

Other incubations:
Add approximately100 µl of the pre-warmed reagent to the section(s), place slide(s) in moisturised incubation chamber and place incubation chamber in a stove (37 °C); check temperature.