

ALP (= alkaline phosphatase) and von Kossa staining

Reagents and solutions to be prepared in advance

1x PBS⁻

weigh in: 8 g NaCl
0.2 g KCl
1.44 g Na₂HPO₄
2.4 g KH₂PO₄

dissolve powder mix in 800 ml dH₂O;
adjust to pH 7.4 with concentrated HCl;
fill up to 1 l with dH₂O;
store at RT;

4% paraformaldehyde

dissolve 4 g paraformaldehyde in 100 ml 1x PBS⁻;
put on stirrer and use moderate heat until the powder has dissolved;
for faster dissolving adjust to pH 8 (using NaOH);
store at 4°C;

0.1 M Tris (base)

dissolve 6.1 g Tris (base) in 500 ml dH₂O;
adjust to pH 9.2 (using concentrated HCl);
store at 4°C;

2.5% sodium thiosulphate

dissolve 1.25 g sodium thiosulphate 50 ml dH₂O;
adjust to pH 9.2 (using concentrated HCl);
store at 4°C;

20% glycerol in PBS⁻

fill 40 ml 1x PBS⁻ into a Falcon tube and add 10 ml glycerol;
store at RT;

Reagents and solutions to be made on the day

ALP reagent mix

= 0.2 mg/ml naphthol AS-MX in 1% N,N-dimethylformamide diluted in 0.1 M Tris (base) pH 9.2
plus 1 mg/ml Fast Red TR

e.g., for 10 ml staining solution:

weigh in 2 mg (= 0.002 g) of naphthol AS-MX (Sigma) into a glass beaker;
add 100 µl N,N-dimethylformamide (toxic! dissolves plastic!);
add 10 ml 0.1 M Tris (base) pH 9.2
weigh in 10 mg (= 0.01 g) Fast Red TR into a centrifugation tube;
add naphthol-Tris solution and mix by inverting the tube several times;
filtrate the ALP reagent mix using a sterile filter;

1% silver nitrate solution

dissolve 100 mg (= 0.1 g) silver nitrate in 10 ml dH₂O;
can **only** be stored **for a few days** at RT!

Staining procedure

carry out on bench:

- 1) wash cells 2x with 1x PBS⁻
- 2) add filtrated ALP reagent mix and leave for 2 min (should go pink)
- 3) wash cells 2x with 1x PBS⁻ (*if just performing ALP staining, proceed to step 11!*)
- 4) add 4% paraformaldehyde and leave for 5 min
- 5) wash once with 1x PBS⁻
- 6) wash once with dH₂O
- 7) add 1% silver nitrate solution and leave on a light box for 10-60 min
- 8) wash 3x with dH₂O
- 9) add 2.5% sodium thiosulphate and leave for 5 min
- 10) wash 2x with dH₂O
- 11) leave in 20% glycerol in 1x PBS⁻
- 12) can be left on bench for a couple of weeks