Detection of mitochondrial DNA using copper ions

Stock solutions to be prepared:

Solution A **- 20 mM $CuSO_4$ and 500 mM NaCl**.

Dissolve $CuSO_4$ in deionized water, add an appropriate volume of 1 M NaCl (stock solution) and fill with deionized water to the final volume. Store at 4°C.

Solution B **- 50 mM sodium ascorbate and 200 mM Hepes pH 7.0.**

Dissolve sodium ascorbate in deionized water, add appropriate volume of 1 M Hepes (stock solution, pH 7.0) and fill with deionized water to the final volume. Store at −20 °C.

**As an aqueous solution of sodium ascorbate is the subject of quick air oxidation, prepare the aliquots in Eppendorf tubes and seal them with parafilm.*

Protocol

1. Fix the samples with 2% formaldehyde in 1× PBS (10 min, room temperature).
2. Wash in 1× PBS (3×).
3. Permeabilise the samples with 0.2% Triton X-100 in 1× PBS (10 min, room temperature).
4. Wash in 1× PBS (3×).
5. Prepare fresh working cleavage mixture: add one volume of the solution A and one volume of the solution B to twenty-three volumes of deionized water just prior to the DNA cleavage. Mix on vortex for 5 s.

**Pre-warm solution A, solution B and water to 20°C before use.*

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6. Remove the buffer from the samples and immediately add the prepared working cleavage mixture.
7. Put the samples on the shaker for 10 s at 300 rpm and 20°C.

**In the case of cells grown on coverslips, perform this step in a Petri dish. Do not perform it on drops of prepared mixture as it protects the sample from oxygen.*

**1 ml of mixture is enough for Petri dish with the diameter of 3.5 cm.*

**Use one coverslip per one Petri dish; otherwise, it is necessary to check the position of the coverslips during the next step to protect their mutual sliding resulting in stacking.*

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8. Remove the working cleavage mixture.
9. Wash the samples with 100 mM Tris– HCl, pH 7 (3×).
10. Prepare fresh solution for enzymatic labelling of mtDNA:

DNA polymerase I (final concentration: 0.2 U/µl), 1× buffer for DNA polymerase I, 0.05 mM dATP, dGTP, dCTP mixture and alternatively biotin-16-dUTP or digoxigenin-11-dUTP or Alexa Fluor 555-dUTP.

**If the signal is low and biotin-dUTP or digoxigenin-dUTP are used, add 0.05 mM dTTP to the solution, it can improve the signal. Do not add dTTP if Alexa Fluor 555-dUTP is used as it can completely eliminate the signal.*

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11. Incubate the samples in the solution for enzymatic labelling for 20 min at room temperature.

**In the case of cells grown on coverslips, this and all additional steps can be performed on drops of solutions.*

**Use 30 μl per circular coverslip with the diameter of 12 mm or 50 μl per well in the case of 96-well plates.*

12. Wash with 25 mM Tris–HCl, pH 7.5 and 150 mM NaCl (3×).
13. **A. If Alexa Fluor 555-dUTP** was used, continue with step 16.

B. If biotin-16-dUTP or digoxigenin-11-dUTP were used, incubate the samples with the primary anti-biotin or anti-digoxigenin antibody diluted in 25 mM Tris–HCl, pH 7.5 and 150 mM NaCl (at least 30 min at room temperature).

**Use 30 μl per circular coverslip with a diameter of 12 mm or 50 μl per well in the case of 96-well plates.*

**In the case of the detection of additional cellular components by specific antibodies, add the required primary antibody to the mixture described in step 13 and secondary antibody to the mixture described in step 15.*

14. Wash the samples four times with 25 mM Tris–HCl, pH 7.5 and 150 mM NaCl.
15. Incubate the samples with the prepared secondary antibody (dilution according to the manufacturer or testing) in 25 mM Tris–HCl, pH 7.5 and 150 mM NaCl and incubate for at least 30 min at room temperature.
16. Wash the samples four times in 25 mM Tris–HCl, pH 7.5 and 150 mM NaCl.
17. In the case of coverslips, remove the buffer by holding each coverslip on its edge with forceps and remove any excess of the liquid from the coverslip by touching its edge on the filter paper. Apply drops of the mounting medium on the glass slide (3 – 5 µl) and carefully lay the coverslips onto the mounting medium. Avoid the creation of any air bubbles. In the case of 96-well plates, aspirate the buffer and add 100 µl of the mounting media.
18. Use nail polish to seal the coverslips and to protect the medium from drying. The well plates and coverslips can be stored at−20°C. In the case of well plates, seal them with parafilm to protect the medium from drying.

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