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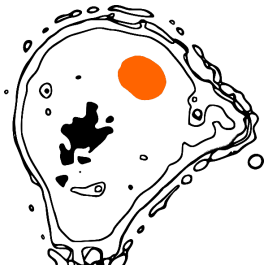
Ookinete purification by centrifugation



In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

This protocol involves working with mouse blood.

Abstract

The ookinetes, as they come from the ookinete culture, are suspended in a solution that also contains blood cells and other parasite stages. Often, it is necessary to purify the ookinetes from these other cell types. This is achieved exploiting biological, chemical, and physical properties of the ookinetes that differ from the other cell types present in the ookinete culture.

This protocol first exploits the ookinete's biological property of laking a Cl^-/HCO_3^- channel in the membrane. On the contrary, the red blood cells (RBC) do have this channel. Because of this, RBC can be selectively lysed using an isotonic solution of NH_4Cl. In solution NH_4Cl dissociates into NH_4^+ and Cl^-, the NH_4^+ then transforms into NH_3, which is permeable to the plasma membrane in contrast to the NH_4^+. Once inside the cell, NH_3 establishes an equilibrium with NH_4^+ resulting in a net influx of Cl^- through the aforementioned ion channel, increasing the NH_4Cl concentration in the RBC considerably and, therefore, of water, causing an osmotic shock and RBC burst. Since HCO_3^- is in equilibrium with CO_2, RBC lysis must be performed during shaking to increase the concentration of CO_2 in the solution (Chernyshev, et al., 2007).

Next a series of differential centrifugations are performed exploiting the sedimentation rate of the ookinetes, which depends on the size, shape, and density of the ookinetes as well as on the viscosity of the medium. The last step involves isopycnic centrifugation in a density cushion taking advantage of the buoyant density of the ookinetes.

This method has been modified from Rodríguez M.C., et al., 2002 and Carter V., et al., 2003.

CITATION

Chernyshev A.V., Tarasov P.A., Semianov K.A., Nekrasov V.M., Hoekstra A.G., and Maltsev V.P. (2007). Erythrocyte Lysis in Isotonic Solution of Ammonium Chloride: Theoretical Modeling and Experimental Verification.. Journal of Theoretical Biology.

LINK

http://dx.doi.org/10.1016/j.jtbi.2007.10.016.

CITATION

Rodríguez M.C., Margos G., Compton H., Ku M., Lanz H., Rodriguez M.H., and Sinden R.E. (2002). Plasmodium berghei: Routine Production of Pure Gametocytes, Extracellular Gametes, Zygotes, and Ookinetes. Experimental Parasitology.

LINK

https://doi.org/10.1016/S0014-4894(02)00035-8

CITATION

Carter V, Cable HC, Underhill BA, Williams J, Hurd H (2003). Isolation of Plasmodium berghei ookinetes in culture using Nycodenz density gradient columns and magnetic isolation.. Malaria journal.

Before start

Materials and equipment:
1.- 15 and 50 ml conical-bottom centrifuge tubes.
2.- Centrifuge, preferably with a swinging rotor and refrigerated to 20°C.
3.- 1 ml pipette and tips.
4.- Transfer pipettes, Pasteur pipettes, or the like to remove the supernatant.
5.- Orbital shaker.
6.- 500 ml sterile Erlenmeyer flask.

Reagents.
1.- Phosphate buffered saline (PBS).
2.- Ammonium chloride (NH_4Cl) at 0.17 M.
3.- Nycodenz (Iohexol or Hiztodenz) at 27.6% in Nycodenz buffer.
4.- Nycodenz buffer (4.95 mM Tris, 0.417 mM EDTA, and 2.95 mM KCl at pH 7.5).

1 Take the ookinete culture form the incubator and mix well to re-suspend the sedimented cells.
Transfer to a 50 ml conical-bottom tube and 1500 rcf, 20°C, 00:03:00

2 Remove supernatant and add NH_4Cl at a ratio of 1:40 in relation to the volume of the pellet.
Transfer the solution into a 500 ml Erlenmeyer flask.

Note

First, add 1 or 2 ml of NH_4Cl and pipette up and down vigorously to disaggregate the pellet. Then add the rest of the solution (about 40 ml in total).

100 rpm, 20°C, 00:25:00

3 Transfer the solution into a 50 ml conical-bottom tube and 725 rcf, 20°C, 00:03:00
.

4 Remove the supernatant and add 2 mL of PBS.
Pipette vigorously to disaggregate the pellet.
Then add 40 mL of PBS.
500 rcf, 20°C, 00:03:00

Note

Ookinetes are very sticky and in the absence of a substrate tend to form clumps that greatly reduce the outcome of the purification.
It is important to pipette thoroughly and to add only one ookinete culture per centrifuge tube.

5 Repeat step 4 but with the following centrifuge conditions: 270 rcf, 20°C, 00:05:00
.

6 Remove supernatant, add 2 mL of PBS, and pipette vigorously.

7 Prepare 5 mL of Nycodenz at 17% with iohexol buffer in a 15 ml conical-bottom tube and slowly add the 2 mL of ookinetes-PBS on the iohexol cushion.
Avoid mixing the two solutions.
895 rcf, 20°C, 00:15:00

8 Remove the brownish interphase between the PBS and the iohexol that contains the purified ookinetes.
Transfer the interphase to a 15 ml conical-bottom and add 10 mL of PBS.

9 270 rcf, 20°C, 00:05:00 to remove the Nycodenz. Repeat two times.

Citations

Chernyshev A.V., Tarasov P.A., Semianov K.A., Nekrasov V.M., Hoekstra A.G., and Maltsev V.P.. Erythrocyte Lysis in Isotonic Solution of Ammonium Chloride: Theoretical Modeling and Experimental Verification.
http://dx.doi.org/10.1016/j.jtbi.2007.10.016.

Rodríguez M.C., Margos G., Compton H., Ku M., Lanz H., Rodriguez M.H., and Sinden R.E.. Plasmodium berghei: Routine Production of Pure Gametocytes, Extracellular Gametes, Zygotes, and Ookinetes
https://doi.org/10.1016/S0014-4894(02)00035-8

Carter V, Cable HC, Underhill BA, Williams J, Hurd H. Isolation of Plasmodium berghei ookinetes in culture using Nycodenz density gradient columns and magnetic isolation.