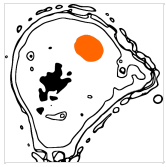

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Affinity purification of ookinetes in coverslips

In 1 collection

Benito Recio Totoro[1,2]

[1]Instituto de Biotecnología - Universidad Nacional Autónoma de México;

[2]Centro de Investigaciones Sobre Enfermedades Infecciosas - Instituto Nacional de Salud Pública

1 Works for me dx.doi.org/10.17504/protocols.io.bmryk57w

Benito Recio Totoro

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 exploits the adhesive and invading characteristics of ookinetes by allowing them to attach to a surface coated with Extracellular Matrix gel (ECM gel). The rest of the cells present in the ookinete culture do not have adhesion properties and can be simply washed away. ECM gel is composed of approximately 1800 proteins, the most abundant are type IV collagen, laminin, entactin, and other proteoglycans. The *Plasmodium* ookinete has several surface proteins that bind to the ECM gel components, such as the P25, and P21/28 protein families that bind to laminin and collagen, and the CTRP, SOAP, and WARP surface or secreted proteins that bind to laminin, and other glycosaminoglycans.

The purification in coverslip allows the obtention of highly pure ookinete cultures that are ready for oocyst culture or further experimentation while still attached to the ECM gel coating.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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COLLECTIONS

Culture and purification of Plasmodium berghei ookinetes

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Part of collection

Culture and purification of Plasmodium berghei ookinetes

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 exploits the adhesive and invading characteristics of ookinetes by allowing them to attach to a surface coated with Extracellular Matrix gel (ECM gel). The rest of the cells present in the ookinete culture do not have adhesion properties and can be simply washed away. ECM gel is composed of approximately 1800 proteins, the most abundant are type IV collagen, laminin, entactin, and other proteoglycans. The *Plasmodium* ookinete has several surface proteins that bind to the ECM gel components, such as the P25, and P21/28 protein families that bind to laminin and collagen, and the CTRP, SOAP, and WARP surface or secreted proteins that bind to laminin, and other glycosaminoglycans.

The purification in coverslip allows the obtention of highly pure ookinete cultures that are ready for oocyst culture or further experimentation while still attached to the ECM gel coating.

BEFORE STARTING

Equipment and materials:
1.- 24-well culture plates.
2.- 12 or 13 mm coverslips.
3.- Point tweezers.
4.- Syringe with needle (make a small bend on the bevel of the needle to create a small hook that is used to lift the coverslips).
5.- 10-100 and 200-1000 µl pipettes and tips.
6.- Inverted microscope.
7.- Incubator at 20°C and at 37°C.

Reagents:
1.- Extracellular Matrix gel (ECM gel or Matrigel).
2.- Phosphate buffered saline (PBS).
3.- Ookinete culture medium: RPMI 1640 medium at pH 8.3 supplemented with 23.81 mM sodium bicarbonate, 0.37 mM hypoxanthine, 25 mM HEPES, 5000 U/ml penicillin, 5 mg/ml streptomycin, 10 mg/ml neomycin, and 20% heat-inactivated fetal bovine serum (Rodríguez, et al., 2002).

Citation: Benito Recio Totoro (12/03/2020). Affinity purification of ookinetes in coverslips . https://dx.doi.org/10.17504/protocols.io.bmryk57w

4.- Oocyst culture medium (optional): Schneider's *Drosophila* medium at pH 6.8 supplemented with 15.87 mM sodium bicarbonate, 20 mM HEPES, 3.68 mM hypoxanthine, 44 µM *para*-aminobenzoic acid, 0.2% lipid/cholesterol solution (Gibco), 100 u/ml penicillin, 100 µg/ml streptomycin, 200 µg/ml gentamycin, and 15% heat-inactivated foetal bovine serum (Al-Olayan, et al., 2002).

Rodríguez MC, Margos G, Compton H, Ku M, Lanz H, Rodríguez MH, Sinden RE (2002). Plasmodium berghei: routine production of pure gametocytes, extracellular gametes, zygotes, and ookinetes. Experimental parasitology.

Al-Olayan EM, Beetsma AL, Butcher GA, Sinden RE, Hurd H (2002). Complete development of mosquito phases of the malaria parasite in vitro. Science (New York, N.Y.).

Purification from cardiac puncture-derived ookinete culture.

1 Thaw an aliquot of ECM gel overnight at **4 °C** in ice.

Keep the ECM gel in ice at all times. ECM gel starts to form a gel at 20°C

2 Place the desired number of clean and sterile coverslips in the wells of a 24-well culture plate.
Coat the coverslips with **12 µl** of ECM gel and allow gel formation at **37 °C** for **00:30:00** .

Avoid spilling the ECM gel outside of the coverslip.
Optionally, the coated coverslips can be left at 37°C until the ECM gel is dry, after which the coverslips can be stored at 4°C. Before use, re-hydrate the ECM gel by adding 500 µl of ookinete medium for 30 min at 37°C. Remove the medium before adding the ookinete culture.

3 Add between **0.5 mL** and **3 mL** of the ookinete culture per coverslip and incubate at **20 °C** for **04:00:00** .

4 15 min before the incubation ends, tilt the culture plate at a 10-15° angle. This will cause the sedimented cells to slide to one side of the container leaving most of the coverslip already free of contaminant cells.

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5 Prepare another 24-well culture plate with **1 mL** of ookinete or oocyst medium.

6 Lift a coverslip with the needle hook and grab it with the tweezers.
While holding the coverslip in a vertical position, wash it with **1 mL** of PBS.
One or two washes is enough to obtain ookinete purities over 85%.

Once the coverslips are removed from the wells and PBS is added, the ookinete culture will start to agglutinate. It is best to dip the coverslip about 1/3 or 1/4 of its diameter into the ookinete culture a couple of times to lose the majority of the contaminant cells before washing with PBS.

7 Transfer the coverslip to the new 24-well culture plate previously filled with medium.

8 Repeat steps 6 and 7 for all the coverslips.

9 The leftover ookinete culture can be used to perform another purification by placing it on new coated coverslips.

Purification from microcultures.

10 This protocol can also be used to obtain purified ookinetes derived from microcultures using only 1 µl of infected blood.
Obtain 1 µl of tail blood of an infected mouse as explained in the *Plasmodium berghei* Ookinete Culture protocol and place in 0.6 Eppendorf tubes with 7 µl of ookinete culture and 1 µl of heparin.
Incubate for 18 h at 20°C.

11 Prepare the coated coverslips as explained in steps 1 and 2.

12 Bring the microculture to 2 ml with ookinete medium and add it to a coated coverslip.

13 Repeat steps 4 to 8 to obtain the purified ookinetes.

Starting from microcultures, approximately 297 ookinetes can be obtained per coverslip.

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