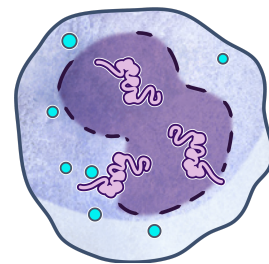


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Opsonization-assisted phagocytosis assay using particles conjugated to synthetic peptides via glutaraldehyde

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We use this protocol and it's working

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Abstract

This protocol describes the opsonization and phagocytosis assay using human leukocytes and synthetic-peptide-decorated particles: *L. floteri*_, RBCs, and yeast. Particle conjugation is achieved through glutaraldehyde crosslinking. Following opsonization with human plasma (positive control) or heat-inactivated plasma (negative control), particle–WBC interactions are measured via microscopy, and the phagocytic index is determined.

Attachments



Opsonization+assay+v..

45KB

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Materials

A. Reagent Preparation

A.1 Prepare 10× RBC lysis buffer (100 ml)

- Ammonium chloride
- Sodium bicarbonate
- Disodium EDTA
- Distilled water

A.2 Prepare 2× HBSS (500 ml)

- NaCl
- KCl
- Na₂HPO₄
- KH₂PO₄
- Glucose
- CaCl₂
- MgSO₄
- NaHCO₃
- Distilled water

A.3 Prepare Wright stain solution (100 ml)

- Wright stain powder
- Methanol

B. Particle–Peptide Conjugation

B.1 Prepare particle suspensions

- *Lactobacillus reuteri* in PBS
- RBCs in PBS
- Yeast in PBS

B.2 Conjugate synthetic peptides

- Glutaraldehyde
- Synthetic peptide
- Glycine

C. WBC Isolation

- Whole blood in CPDA-1 tubes
- 1× RBC lysis buffer

D. Cell Viability and Counting

- WBC suspension
- Trypan blue

E. Plasma Treatment

- Pooled human plasma

F. Opsonization of Particles

A. Reagent Preparation (Estimated time: 30–40 min, excluding storage)

- 1 Prepare 10× RBC lysis buffer (100 ml)
 - 1.1 Weigh 0.82 g ammonium chloride.
Weigh 0.84 g sodium bicarbonate.
Weigh 0.37 g disodium EDTA.
Add all solids to a 100 ml volumetric flask.
Add distilled water to reach 100 ml total volume.
Mix until completely dissolved.
Store at 4 °C for up to 6 months.
- 2 Prepare 2× HBSS (500 ml)
 - 2.1 Weigh: 8 g NaCl; 400 mg KCl; 48 mg Na₂HPO₄; 60 mg KH₂PO₄; 1 g glucose; 140 mg CaCl₂ (anhydrous); 98 mg MgSO₄ (anhydrous); 350 mg NaHCO₃.
Add all solids to a 500 ml volumetric flask.
Add distilled water to 500 ml total volume.
Mix until dissolved.
Store at 4 °C for up to 1 month.
- 3 Prepare Wright stain solution (100 ml)
 - 3.1 Weigh 300 mg Wright stain powder.
Add to 100 ml methanol.
Mix until dissolved.
Store at room temperature in a dark bottle.

B. Particle–Peptide Conjugation via Glutaraldehyde (Estimated time: ~1.5 h)

- 4 Prepare particle suspensions
 - 4.1 Suspend *Lactobacillus reuteri* in PBS at $\sim 1 \times 10^8$ cells/ml.
Suspend RBCs in PBS at $\sim 1 \times 10^8$ cells/ml.
Suspend yeast in PBS at $\sim 1 \times 10^8$ cells/ml.
- 5 Conjugate synthetic peptides



- 5.1 Add glutaraldehyde (stock: 25%w/v or 2.5M) to each suspension to a final concentration of 0.1% (v/v) (10.6mM).
Incubate 30 min at room temperature.
Add synthetic peptide to each suspension to a final concentration of 40 µg/ml (stock: 400 µg/ml).
Incubate 30 min at room temperature.
Quench with glycine to 100 mM final concentration (stock: 1M).
Incubate 10 min at room temperature.
- 6 Wash and resuspend
- 6.1 Centrifuge at 300 × g for 5 min.
Discard supernatant.
Resuspend pellet in PBS.
Repeat wash twice more.
Resuspend final pellet in HBSS or assay buffer.

C. WBC Isolation (Estimated time: 40–50 min)

- 7 Lysis of RBCs
- 7.1 Collect whole blood in CPDA-1 tubes.
Centrifuge at 300 × g for 5 min to separate plasma.
Transfer plasma to a fresh tube and place on ice.
Transfer 1 ml RBC pack with buffy coat to a new tube.
Add 10 ml of 1× RBC lysis buffer.
Incubate 10 min at room temperature with gentle rocking.
Centrifuge 5 min at 300 g.
Discard supernatant.
Repeat lysis if RBCs remain visible.
- 8 Washing and resuspension
- 8.1 Wash WBC pellet once with HBSS.
Centrifuge 5 min at 300 g.
Resuspend WBCs in HBSS or assay buffer.

D. Cell Viability and Counting (Estimated time: 10–15 min)

- 9 Mix 100 µl WBC suspension with 100 µl 0.4% trypan blue.

Incubate for 3 min at room temperature.
Load 100 μ l onto hemocytometer.
Count viable (unstained) and non-viable (blue-stained) cells.

E. Plasma Treatment (Estimated time: 40 min, including incubation)

- 10 Thaw pooled human plasma at 37 °C for 10 min.
Keep plasma on ice until use.
For negative (complement-inactivated) control, heat-inactivate plasma at 56 °C for 30 min in a heat block.

F. Opsonization of Particles (Estimated time: 50 min)

- 11 Prepare particle suspensions at $\sim 1 \times 10^8$ particles/ml.
Add 300 μ l particle suspension to 1.2 ml plasma.
Incubate 30 min at 37 °C with shaking.
Centrifuge 15 min at 1,600 g.
Discard supernatant.
Resuspend pellet in 300 μ l HBSS.

G. Phagocytosis Assay (Estimated time: 50–60 min)

- 12 Prepare WBC suspension at 5×10^6 cells/ml in HBSS.
Mix 200 μ l WBC suspension with 200 μ l opsonized particle suspension.
Incubate 20 min at 37 °C with rotation at 8 RPM.
Centrifuge 8 min at 250 g.
Discard supernatant.
Wash pellet with HBSS.
Repeat wash twice more.
Resuspend final pellet in ice-cold HBSS.
Place 100 μ l suspension onto microscope slide.
Air-dry in a laminar flow hood.
Stain with Wright stain according to manufacturer's instructions.
Air-dry again.

H. Microscopy and Data Collection (Estimated time: varies; ~15–20 min per sample)

- 13 Examine slides under 1000 $\times$ magnification.
Count 50 neutrophils per condition.
Record the number of neutrophils containing ≥ 1 particle.
Record the number of particles per positive cell.
Calculate the phagocytic index: Phagocytic Index = (% positive neutrophils) $\times$ (mean particles per positive cell)



Protocol references

The Drevets, Canono, and Campbell (2015) protocol describes macrophage ingestion and killing assays, including distinguishing bound vs. internalized bacteria using fluorescence microscopy. Boero et al. (2021) use flow cytometry to evaluate neutrophil phagocytosis of *S. aureus*, *employing opsonization and fluorescence readouts for higher throughput*.

Drevets DA, Canono BP, Campbell PA. Measurement of Bacterial Ingestion and Killing by Macrophages. *Curr Protoc Immunol* 2015;109:14.6.1-14.6.17. <https://doi.org/10.1002/0471142735.IM1406S109>.