



Feb 24, 2025

High-throughput 96-well plate-based porcine antibody isolation protocol

 [PLOS One](#)

✓ Peer-reviewed method

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvmee6ng3p/v1>

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PLOS ONE Lab Protocols

Spotlight series



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Protocol Citation: John Byrne, Christina Bourne, Sitka Eguiluz, Stephanie Langel, Elisa Crisci 2025. High-throughput 96-well plate-based porcine antibody isolation protocol. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.yxmvmee6ng3p/v1>

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Protocol status: Working

We use this protocol and it's working

Created: June 05, 2024

Last Modified: April 28, 2025

Protocol Integer ID: 101284

Keywords: Antibody, Pig, Swine, Porcine, Resin, Chromatography, IgA, IgG, Isolation, High Throughput, based porcine antibody isolation protocol, porcine antibody isolation protocol this protocol, iga from pregnant, variety of immunoassay, immunoassay, lactating swine, modified column chromatography technique, column chromatography technique, consistent isotype yield from individual animal, igg, individual animal, iga

Funders Acknowledgements:

Bill and Melinda Gates Foundation

Grant ID: INV-22595D

Abstract

This protocol describes a modified column chromatography technique to isolate both IgG and IgA from pregnant/lactating swine to use in a variety of immunoassays. This high throughput method allows for both a rapid and consistent isotype yield from individual animals and different tissue samples.



Materials

Glycine, 0.2M buffer soln., pH 2.5 Cat# J61855.AP (Thermo Scientific)
Tris Hydrochloride, 1M Solution (pH 8.0/Mol. Biol.) Cat# BP1758-500 (Fisher Scientific)
Ethanol, Absolute (200 Proof), Molecular Biology Grade Cat# BP2818500 (Fisher Scientific)
Tris-EDTA, 1x Solution, pH 8.0 $\pm$ 0.1 Cat# 77-86-1 (Fisher Scientific)
Sodium chloride, ACS, 99.0% min Cat# 7647-14-5 (Fisher Scientific)
Pierce Protein G Agarose Cat# 20399 (Thermo Scientific)
Peptide M / Agarose Cat# gel-pdm-5 (InvivoGen)
CaptureSelect IgA-XL Affinity Matrix Cat# 2943972010 (Thermo Scientific)
Ultra-pure water - In House
Corning Mediatech Cell Culture Phosphate Buffered Saline (10X) Cat# MT-46013CM (Fisher Scientific)
Universal 200ul Pipette tips Cat# 76322-144 (VWR)
BrandTech Scientific 781722, 96-Well Plates, immunoGrade, Clear Transparent, Flat Bottom Cat# 91-415F (Genesee Scientific)
Multiscreen 96 well Plate, hydrophobic PVDF membrane Cat# MSIPS4W10 (Millipore Sigma)
Thermo Scientific NanoDrop 2000c Spectrophotometer
Sorvall Legend XTR (Refrigerated), 120V (Thermo Scientific)
Sorvall Legend Micro 21R Microcentrifuge (Thermo Scientific)

Before start

Prepare 1x PBS
Prepare 0.1M Glycine
Prepare 70% ETOH
Prepare Ab Binding Buffer (10mM TRIS, 1mM EDTA, 300mM NaCl)



Sample Preparation

1m

- 1 Quickly vortex all samples to ensure no clotting from serum and no fat blockages from milk
- 2 Centrifuge all samples at Room temperature for 2000 x g, Room temperature, 00:01:00
- 3 Add 100 μ L sample (serum/milk/colostrum) to 100 μ L Ab Binding Buffer
- DO NOT ADD ANY PRECIPITATE TO THE MIX

1m



Resin Plate Preparation

3m

- 4 Remove the Multiscreen 96 well ELISpot Plate from packaging
- 5 Place the Multiscreen 96 well ELISpot Plate on top of a 96-Well immunoGrade ELISA Plate ensuring the ejection ports of the ELISpot plate are in line with the ELISA plate wells
- 6 Add 50 μ L Protein G, CaptureSelect IgA, or Peptide M to each well
- ONLY USE ONE RESIN PER WELL
- 7 Wash all wells by adding 50 μ L 70% ETOH to each well
- 8 Centrifuge the plate stack at 4000 x g, Room temperature, 00:03:00
- 9 Discard flow through from the bottom plate
- 10 Wash all wells by adding 200 μ L 1 $\times$ 7.4pH PBS to each well
- 11 Centrifuge the plate stack at 4000 x g, Room temperature, 00:03:00



3m



3m





12 Discard flow through from the bottom plate

13 Restack the plates

Antibody Isolation

41m

14 Add  100 μ L Sample (serum/milk/colostrum) / Ab Binding Buffer mix per well to each plate 

15 Place the plate on a plate shaker and shake  600 rpm, Room temperature , 00:30:00

30m



16 Remove the plate stack from the shaker and centrifuge the plate at  4000 x g, Room temperature, 00:03:00

3m



17 Discard the flow through from the bottom plate

18 Restack the plates

19 Wash all wells by adding  200 μ L Ab Binding Buffer to each well



20 Centrifuge the plate stack at  4000 x g, Room temperature, 00:03:00

3m



21 Discard the flow through from the bottom plate

Antibody Isolation

41m

22 Repeat Steps 19-21 3 times



Antibody Isolation

41m

23 Place a new 96-Well immunoGrade ELISA Plate under the Multiscreen 96 well ELISpot Plate to ensure that collected antibody are pure



24 Add  100 μ L 0.1M Glycine and incubate  00:02:00 , Room temperature

2m



25 Elute the antibodies by centrifuging the plate stack at  4000 x g, Room temperature, 00:03:00

3m



26 Repeat step 24 and 25

27 Add  20 μ L 1M TRIS to each well to neutralize the solution



Antibody Concentration and Storage

11m

28 Label one Amicon ultra 0.5 50k Centrifuge filter for each sample

29 Remove the  220 μ L glycine-TRIS solution from the well and place into the corresponding Amicon ultra 0.5 50k Centrifuge filter



30 Centrifuge the filter tubes at  12000 x g, Room temperature, 00:05:00

5m



31 Wash each sample with  400 μ L 1 $\times$ 7.4pH PBS



32 Centrifuge the filter tubes at  12000 x g, Room temperature, 00:05:00

5m



33 Label one Amicon ultra 0.5 50k Centrifuge collection tube to store each sample

34 Invert the Amicon ultra 0.5 50k Centrifuge filter into the new labeled storage tube



35 Centrifuge the filter tubes at  1000 x g, Room temperature, 00:01:00
- this should yield ~ 100ul of isolated antibody

1m



36 Remove the filter from each tube and store isolated antibodies at  4 °C until later use

Quantify Antibody concentration

37 Use NanoDrop, select the "Protein A280" program

38 Pipette  1 µL isolated antibody on the NanoDrop pedestal



39 Analyze the sample



40 Record published mg/mL