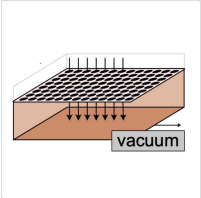

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Scaled High Throughput Vacuum PhIP Protocol

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protocol .

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This protocol was developed as a high throughput, low cost technique to perform phage display immunopreceipitation. Here we overview all the steps, from isolating antibodies from a complex matrix of patient sample, washing the sample and amplify targets through two rounds of selection.

3D printable adaptor schematic compatible with Arctic White 96 well filter plates can be found attached and can be downloaded from the NIH 3D Print Exchange (Model ID 3DPX-016424).

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Sabrina A Mann, Sara Vazquez, Gavin Sowa, anthea.mitchell , Caleigh Mandel-Brehm, Joe DeRisi 2022. Scaled High Throughput Vacuum PhIP Protocol. **protocols.io** https://protocols.io/view/scaled-high-throughput-vacuum-phip-protocol-btnznmf6

phage display, immunoprecipitation, phage

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Equipment Needed and Part Numbers:

- Vacuum Manifold- Qiagen Cat. No 19504
- Plate Sealer- Applied Biosystems Cat No: 43-331-83
- Foil seals- Bio-Rad Cat No: MSF1001
- Gas permeable seal- 4titude Cat No: 1149R80
- 96 well filter plate- Arctic White Cat No: AWFP-F20022
- Round 96 well 2ml deep well plate- Genesee Science Cat No: 22-485
- Square 96 well 2ml deep well plate- VWR Cat No: 75870-796
- 12 span 1000uL multichannel pipet
- 3D printed plate holders- see attached schematic which can also be downloaded from the NIH 3D Print Exchange (Model ID 3DPX-016424).

Reagents Needed:

- Protein A/G Beads
- 1xTBS-Tween
- BSA Fraction V
- LB-Carb
- BLT5403
- Phage Display Library
- RIPA
  -10mM Tris-HCL (pH 7.4)
  -1.0% Triton X
  -0.1% SDS
  -140mM NaCl
- TNP40
  -10mM Tris-HCl (pH 7.4)
  -140mM NaCl
  -0.1% NP40 (Tergitol)
- SM Buffer
  -50mM Tris-HCl (pH 7.4)
  -100mM NaCl
  -10mM MgSO4+7h2O anhydrous (powder)

:

Before getting started you will need a titered phage display library, patient samples and all required materials mentioned in this protocol.

Day 1- Blocking Plates

1 Make blocking solution to prevent sample and library from binding to plates, this step also helps with movement of protein A/G beads later on.

Blocking solution (make fresh for every experiment):
3.0% BSA Fraction V
1x TBS-Tween
Mix thoroughly and filter through a 0.22um filter

2 Aliquot blocking solution into Round-bottom 96 well 2ml deep well plates according to the number of wells and plates needed for experiment for both rounds of IPs. Seal tightly with foil seal.
Example: If running 4 plates worth of samples (384 samples including controls), block 8 plates, 4 for each round.

NOTE: Highly recommend to do an even number of plates, and only do 2-4 plates worth of samples when running this protocol for the first time. The maximum number of plates is about 6 plates if running the protocol solo, and 8 plates if there is a second person to help during the immunoprecipitation washes.

3 Incubate at **4 °C** inverting overhead or rocking on platform at minimum **Overnight** or 4h for a minimum **04:00:00** at **Room temperature** . Solution can block for several days at **4 °C** maximum.

Day 1- Setting up IPs

4 After plates have sufficiently blocked, unseal plates and discard all blocking solution contained in one set of IP plates by dumping the contents into the sink and gently tapping on clean paper towels. Continue blocking the other set of plates for round two of IP.

5 Add **500 µL** of phage library per well. If a canary well is desired (to control for phage contamination of reagents and during the protocol) add SM buffer to that well instead of phage library.

6 Add sample to library, amounts vary depending on sample type and concentration of IgG in sample:

1 µL of serum OR plasma in 1x Storage Buffer

10-20 µL of CSF in 1x Storage Buffer

1 µg of Commercial Antibody (for positive control, recommend to put one of these on each plate randomized location.

A highly recommended sample type is an AG control well ("mock-IP") with no sample to control for nonspecific binding of phage library to A/G beads. Recommended to put 2 - 4 of these on each plate and randomize the location.

7 Seal plate very well (should be able to see outline of the individual wells) with foil seal and plastic plate sealer tool. Then incubate by inverting overhead or on rocker platform at **4 °C** **Overnight** (minimum of 6 hours, maximum of 18 hours)

8 On a clean, phage-free lab bench, prepare an overnight starter culture of E.coli (BLT5403) as a working stock from a frozen glycerol stock in LB-Carb.

Day 2- Preparing AG Beads

9 **Prepare A/G Beads:**
NOTE: handle the beads gently using wide bore tips if possible to prevent damaging the beads.

1. Fully re-suspend beads by gently flipping by hand or placing on overhead rotator.
2. Calculate amount of beads needed. For the peptidome library we use **10 µL** of A and **10 µL** of G beads per reaction, however some prefer to use **20 µL** of A and **20 µL** of G per reaction to increase the capture of IgG and IgA.
3. Mix packed Protein A and Protein G beads in a 1:1 ratio.

10 Aliquot amount of beads needed into a container able to hold more twice the volume of beads. 5m
We typically use a 5mL eppendorf tube or 15mL conical tube. Add 1x bead volume of cold TNP40 and mix overhead for **00:05:00** at **4 °C** or until fully resuspended, then place on magnetic rack until fully pelleted.

11 Remove supernatant and immediately add cold TNP40 again. Mix overhead again. Repeat this wash step a total of three times to fully remove the solution that the beads were stored in.

12 On the final wash step, elute the beads in enough TNP40 for the number of reactions needed. From experience, eluting in **25 µL** per reaction works well to form a nice droplet with the wide bore tips when adding beads to the peptidome and sample.

Day 2- Adding Beads to Sample Bound to Library Targets 5m

13 2m
1. Spin plates from overnight incubation at **800 rpm** for **00:02:00** to remove all liquid off from the top of the seal.
2. Gently remove seal.

14 1h
1. Put washed A/G beads into a reservoir container.

2. Add A/G beads to sample/library plate with wide bore tips using a multichannel quickly, making sure the beads stay resuspended and do not settle on the bottom of the reservoir.
3. Seal plate with foil seal.
4. Overhead mix for **01:00:00** at **4 °C** .

NOTE: Don't exceed 1 hour, more time will increase nonspecific binding. (50min-1hr of bead binding is okay; start first group of plates at 50 mins)

15
1. Simultaneously, prepare new E.coli culture by growing 1-3mL of the overnight culture in enough LB-Carb (0.5mL for each sample).
2. Prepare vacuum plates with labels and vacuum manifold.
3. Recommend to label plates as Plate 1 Round 1, Plate 2 Round 1, etc. to keep track steps during the immunoprecipitation washes.

Day 2- Immunoprecipitation Washes 1m

16 40s

NOTES:
1. Keep RIPA on ice to help keep samples cool during washes
2. Plate spins should be at **800 rpm** **00:00:20** on a tabletop centrifuge as we have found that this is the minimum amount of time and speed required to remove liquid from the top of seal, however we recommend to test time on own centrifuge.
3. After hour is up, spin 2 plates (first set if doing more than 2 plates total) at **800 rpm** **00:00:20**

17
1. Using a 12-channel P1000 multichannel, gently mix and transfer **500 µL** of mixture to the corresponding row on labeled **96 well filter plate** that is placed on the vacuum manifold with the vacuum **OFF**. Repeat with all rows keeping the same layout and using fresh tips on each row.
2. Once all rows are transferred, carefully turn vacuum on to HALF POWER and let supernatant just drain through.
3. Turn vacuum off, GENTLY/SLOWLY release the vacuum pressure to avoid tearing the filter membranes. Using a multichannel, add **500 µL** of RIPA from a reservoir (empty tip box works great) to each well, hovering above the wells to use the same tips for the entire plate. If you accidentally touch tips to plate, discard and use new tips.

18 17m
1. Seal top of plate VERY WELL with foil seal and sealer.
2. Carefully tap plate on manifold a few times then blot three times on CLEAN paper towels. Then place in plate holder. The plate should notch in but not be too snug.
3. Place plate on rocker for **00:07:00** - **00:10:00** such that the liquid is freely moving in the wells.
4. If doing more than one set of plates, begin on the next set of two plates while the first pair incubates on the rocker.

19 1. After **00:07:00** - **00:10:00** , spin the plates for **800 rpm** for **00:00:20** 17m 20s
2. Place on vacuum manifold and turn on vacuum to half power, let liquid just drain through, turn off the vacuum and add **500 µL** RIPA to each well while hovering above the wells.

20 Repeat steps 18-19 three more times for a total of 5 washes.
NOTES:
- Each addition of RIPA counts as a wash.
- Aim for no more than 2 hours for the 5 washes such that the sample is not in RIPA for too long.
- When doing multiple plates, batch them in pairs for easy centrifugation. i.e. six plates would be done in three pairs
- Labeling the sides of each plate with checkboxes for each wash is helpful for keeping track of washes per plate.

Day 2- Lysis/Amplification

21 1. After final/5th wash drain liquid, turn off and release vacuum pressure, and add 150uL LB-Carb to each well.
2. Using multichannel mix one row until all beads resuspended and transfer to a new **Square 96 well 2ml deep well plate**.
3. Repeat with fresh tips on each row.

NOTE: Be sure to get all beads off the filter and transferred to new plate. It is fine to introduce bubbles at this step.

4. Add **500 µL** E.coli at OD600 0.3-0.6 to the bead/LB mixture.

NOTES:
- If doing multiple plates, try to add the E. coli to the LB around the same time so lysis occurs simultaneously.
- An OD on the lower side of that range is perfectly fine and will help with preventing the filters from clogging on round two.

22 1. Seal with a gas permeable seal, and incubate in shaker incubator for 1-2 hrs.
NOTES:
- Catch the clarification as soon as possible!
- Compare lysis to a canary well (can be on a completely separate plate or tube) which should remain cloudy while the wells containing phage library clarify when on magnet to pellet beads.
- Once the wells look clear, proceed immediately to next step. Do not let it incubate for longer than necessary, as this will decrease phage titers.

23 Add **5.0 Molarity (M)** Sodium chloride **P212121** to each well for a final molarity of **0.5 Molarity (M)** in the clarified E. coli culture (volume changes with amount of lysate, normally around 60-65uL).

Day 3- Preparing for Round 2 30m

24 1. Spin plate at **4 °C** for **00:30:00** at **3220 rcf** (or max speed) to remove E. coli cell 30m

debris.

2. While plate is spinning, empty out blocking solution from second round of plates.
3. After spinning, use the Integra or a multichannel to transfer **200 µL** from the top of the wells, avoiding the cell debris at the bottom, to the previously blocked plates.
4. Add **300 µL** of SM Buffer per well.

NOTE: This is to dilute the lysate, so it doesn't clog the filters for round 2.

1. NOTE: Save Round 1 lysates and store at **4 °C** as a backup in case round 2 goes poorly or if you wish to sequence Round 1.

25

1. Add samples in same volume and configuration as done in starting Round 1 to plates.
2. Repeat IP one more time for a total of 2 rounds of immunoprecipitation. One can do additional rounds of IP for more selection if desired.
3. Store final lysate short term at **4 °C** or long term at **-20 °C**
4. To prepare samples for sequencing, follow with protocol below:

Phage Display Library Prep Method
by Sabrina Mann,
Chan Zuckerberg Biohub

PREVIEW RUN