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

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CODEX Antibody Conjugation Protocol

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ABSTRACT

Used by the Snyder Lab at Stanford University for CODEX for human intestine.

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PROTOCOL integer ID: 98091

1 Block non-specific absorption of antibody onto 50k MWCO spin column with PBS-tween (0.2% tween solution) by adding 500 uL into the top of each column (rinse all areas of filter) and spinning down at 12,000g for 2 minutes

2 Remove all liquid from the top of the column and discard flow-through. Label each column and filter.

3 Spin down the antibodies

4 Optional: measure the concentration of your antibodies

4.1 Nanodrop, protein A280, blank with PBS, IgG

5 Add 100 ug of antibody to the top of each pre-blocked 50k MWCO filter columns. If the volume is less than 100 uL, add 200 uL of PBS to the column before adding the antibody

6 Spin column down at 12,000g for 8 minutes. Discard flow-through.

6.1 If the volume of antibody needed did not fit, repeat this step until all of the antibody is contained in the filter unit.

7 Optional: If antibody needs to be washed (Azide/BSA) add 400 uL more of PBS and spin down again at 12,000g for 8 minutes. Repeat as necessary

8 Create the following solution and vortex to mix: 5 uL 500mM TCEP, 5 uL 500mM EDTA, 990 uL 1X PBS

9 Add 360 uL of this solution to the top of the MWCO filter unit. Pipette mix then briefly vortex the tube

10 Incubate at RT for 30 minutes (do not go longer than 30 minutes)

11 Spin the solution at 12,000g for 8 minutes. Discard flow-through

12 Add 400 uL Buffer C and spin the solution at 12,000g for 8 minutes. Discard flow-through.

12.1 Remove oligo aliquots from the freezer at this point

13 Make high salt buffer C: 380 uL Buffer C, 20 uL 5M NaCl

14 Solubilize oligos. Oligo:antibody ratio is 2:1. For 100 ug antibody use 200 ug oligo. Do one tube at a time. Once oligo is solubilized the next step should follow immediately. Add high salt buffer C to the tube containing the top oligo. Mix with pipette until oligo is completely solubilized. There should be no visible cloudiness to the solution.

14.1 PCR tubes (100 ug/tube): use 2 PCR tubes, add 200 uL high salt buffer C to each

14.2 Large tube (lyophilized) (200 ug/tube): add 400 uL high salt buffer C

14.3 Large tube (prediluted) (200 ug/tube): add high salt buffer C to a total of 400 uL

15 Add entire oligo solution to the top of the filter column containing the the reduced antibody solution.

16 Mix well with the pipette. Do not vortex.

17 Incubate the oligo-antibody mix for 2h at room temperature (can go longer than 2 hours)

18 Spin the column down at 12,00g for 8 minutes. Discard flow-through.

19 Add 450 uL of high salt PBS to the top of the column and spin down at 12,000g for 8 minutes. Discard flow-through.

19.1 High salt PBS (1M NaCl PBS): 5 M NaCl diluted to 1M in PBS

20 Add another 450 uL of high salt PBS to the top of the column and spin down at 12,000g for 8 minutes. Discard flow-through.

21 Repeat step #20 one more time.

22 Add CODEX antibody stabilizer solution to the top of the column in a 2:1 ratio (2 uL solution : 1 ug antibody)

22.1 CODEX antibody stabilizer (can make stock and store at 4C for a few weeks): 900 uL antibody stabilizer (BOCA scientific, + 0.2% sodium azide), 10 uL 0.5M EDTA, 100 uL 5M NaCl

23 Invert into new collection tube. Spin down at 3,000g for 2 minutes. KEEP COLLECTED SOLUTION

24 Combine total volume of conjugated antibody for a given antibody into a single screw-top tube and store at 4C — DO NOT FREEZE