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CODEX Antibody Conjugation Protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

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



- 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