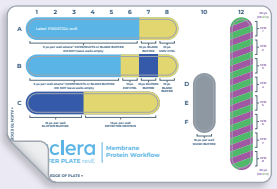
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Membrane Protein Workflow - Transfer Plate and Beads Preparation Quick Start

This guide is for setting up and preparing the transfer plate which will be used to load reagents into the cartridge before you start your run. The guide is for use when working with membrane proteins or when you want the purify-all workflow.

Apply the label provided with the Membrane Protein Cartridge Reagent Kit **NC3013** to a 96-well plate and peel off pre-cut sections corresponding to rows A, B and C, and columns 10 and 12 of the transfer plate.

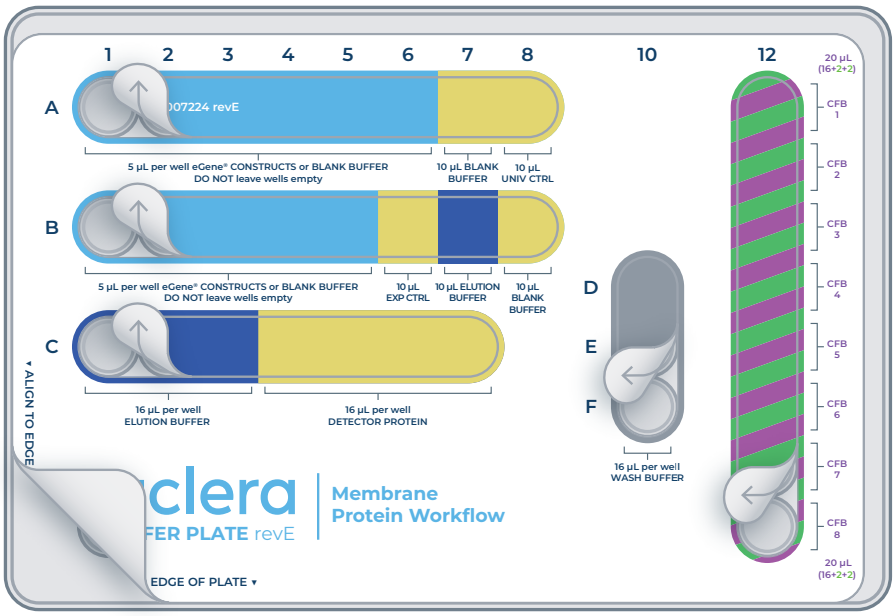


eGene™ DNA constructs:

Color	Description	Well #
	5 µL	A1-A6
	5 µL	B1-B5

Reagents from NC3013:

Color	Description	Well #
	10 µL of Blank Buffer	A7 and B8
	10 µL of Universal Control	A8
	16 µL of Detector Protein	C4 - C7
	10 µL of Expression Control	B6
	16 µL of Wash Buffer	D10, E10 and F10
	10 µL of Elution Buffer	B7
	16 µL of Elution Buffer	C1 - C3
	16 µL of Cell-free Core	12A - 12H
	2 µL of Additive 1	Mix these reagents with Cell-free Core at cartridge loading step
	2 µL of Additive 2	



Transfer plate



Important note: Keep the transfer plate and the screen reagents from the Membrane Protein Cartridge Reagent Kit **NC3013-1** on ice.

Strep Purification Beads

Important note: Strep Purification Beads are provided in 1x 400 µL or 2x 200 µL aliquots of 5% v/v suspension and must be stored at +4°C.

1. Take an empty 1.5 mL Eppendorf tube.
2. Take the 1x 400 µL or 2x 200 µL vial(s) of Strep Beads from the fridge and give it/them a quick spin for 2 seconds in a microcentrifuge to pellet the beads.
3. a. **If working with 1x 400 µL aliquot**, set a p1000 pipette to 300 µL. Resuspend the beads by gently pipetting up and down 10 times. Then, WITHOUT PAUSING, pipette up an 11th time and transfer 300 µL to the empty 1.5 mL Eppendorf tube.
   b. **If working with 2x 200 µL aliquots**, set a p200 pipette to 150 µL. Resuspend the beads by gently pipetting up and down 10 times. Then, WITHOUT PAUSING, pipette up and transfer 2x 150 µL (300 µL in total) to the empty 1.5 mL Eppendorf tube.
4. Place the tube with Strep Beads on a magnetic rack and capture for 30 seconds.
5. Remove all the supernatant with a p1000 pipette and discard the liquid.
6. Remove the tube with Strep Beads from the magnetic rack. Resuspend the beads in 300 µL Wash Buffer by slowly pipetting up and down 10 times.
7. Repeat steps 4 to 6 twice more for a total of three washes.
8. After the third wash, spin down the tube and place it back on a magnetic rack and capture for 30 seconds.
9. Remove all the supernatant from the tube with a p200 pipette and discard the liquid.
10. Spin down the tube, place it back on a magnetic rack and remove the residual buffer with a p20 pipette, being very careful to not take up any beads.
11. Remove the tube and add 35 µL of Wash Buffer to the bead pellet to make 30% v/v Strep beads working solution.
12. With a p20 pipette set to 15 µL, resuspend the beads by gently pipetting up and down 10 times. Then, WITHOUT PAUSING, pipette up an 11th time and take off a 15µL aliquot and transfer to an Eppendorf tube.
13. Repeat resuspension and aliquoting steps twice more to create a total of three 30% v/v Strep Beads working solution aliquots.
14. Keep the beads in the tubes on the bench, NOT ON ICE. **The beads should not be loaded onto the transfer plate.**
15. Using a p20 pipette, mix the Strep Purification Beads tube by gently pipetting up and down 10 times, avoiding air bubbles, and then immediately pipetting up an 11th time to collect 12 µL of magbeads. Immediately dispense into port A10 of the cartridge.
16. Repeat the same process for the second and third tubes, dispensing into ports B10 and C10, respectively.

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