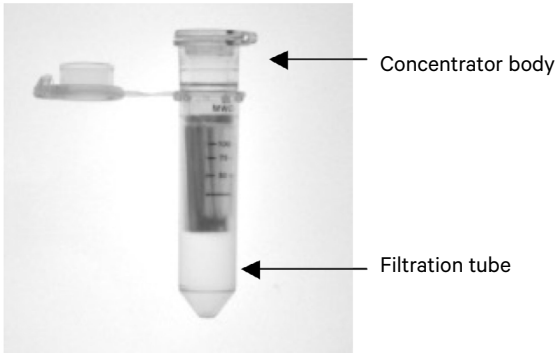
SUPPLEMENTAL PROTOCOL

Concentration of Dilute Antibody Solutions

Together we breakthrough™

The HRP-Antibody All-in-One™ conjugation protocol requires the initial antibody concentration to be at 4–5 mg/ml and 25 µl. Many antibody vendors package their products at significantly more dilute concentrations (e.g. 0.2 to 1.5 mg/ml). In these instances, the antibody sample will need to be concentrated to ~5 mg/ml and 25 µl before starting. The All-in-One kit provides two-diafiltration apparatus (30 kDa MWCO) for this purpose (see Figure below). Follow the procedure below to avoid antibody loss or aggregation on the membrane surface.

Note: Dilute antibody solutions require at least 125 µg of starting antibody (e.g. 500 µl at 0.25 mg/ml) since diafiltration recovers about 80% of input antibody. If there is sufficient material, we recommend the antibody concentration be confirmed using a Bradford protein assay.



Diafiltration apparatus used for concentrating dilute antibody samples prior to starting the All-in-One conjugation protocol.

Antibody Concentration Protocol

Note: The diafiltration apparatus are made to contain and process a maximum volume of 500 µl. If a volume greater than 500 µl is to be concentrated, multiple loadings will be required.

1) Open the cap of the diafiltration concentrator.
2) Transfer a maximum of 500 µl of dilute antibody solution (equivalent to 125 µg antibody) to the concentrator.
3) Close the cap and orient the apparatus in the centrifuge so that the volume markers face toward the center of the centrifuge rotor. Use an appropriate balance tube opposite the apparatus.
4) Centrifuge for 2 minutes at 5,000 x g.
5) Visually estimate the remaining volume in the concentrator body. If the volume remaining in the concentrator body is greater than 25 µl, gently pipet the solution up and down with a pipette to mix. Be careful not to touch or scrape the filter membrane surface during this step.
6) Repeat steps 4 and 5 until the volume in the filter cup reaches the 25 µl mark.
7) Transfer the concentrated IgG solution (25 µl at 4-5 mg/ml) to a 1.5 ml microcentrifuge tube and proceed with the procedure.

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