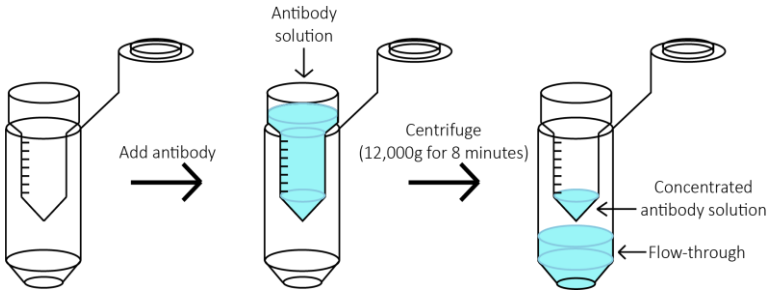
Conjugating CODEX® tags on antibodies of choice

1. Prepare Conjugation Reagents and Antibodies

a. Purify antibodies if needed (see User Manual)
b. Measure and calculate concentration of Antibodies using pre-set IgG settings of Nanodrop
c. Calculate the volume of solution corresponding to **50 µg of antibody**.
d. Retrieve the following reagents now:
   - Reduction Solutions 1 & 2
   - Filter Blocking Solution
e. Retrieve the following reagents in ~1 hour:
   - Conjugation Solution
   - Barcodes
f. Retrieve the following reagents in ~3 hours:
   - Purification Solution
   - Antibody Storage Solution



Antibody stocks:

- Purified Antibodies in PBS or in a similar buffer. Antibodies should be free of carrier proteins, such as BSA, and other chemicals such as gelatin or glycerol.

Akoya Materials

- CODEX® Conjugation Kit
  - Reduction Solution 1
  - Reduction Solution 2
  - Filter Blocking Solution
  - Conjugation Solution
  - Purification Solution
  - Antibody Storage Solution
- CODEX® Barcodes

Additional Materials

- 50kDa MWCO filter
- 1.5 ml sterile tube(s)
- 1.5 ml low bind tubes
- ddH2O
- 0.2 ml PCR tubes
- Ice bucket
- Centrifuge for 1.5 ml tubes
- NanoDrop

2. Reduce Purified Antibody

a. Label a 50kDa MWCO filter for each antibody.
b. Add **500 µl** of **Filter Blocking Solution** to the top of **each 50kDa MWCO filter**.
c. Spin down at **12,000g** for **2 mins**.
d. Remove all liquid; discard both the liquid left on the top of the column and the flow-through solution. Use a pipette if necessary to remove all liquid on top of the column.
e. Add **50 µg** of the **antibody** in a volume **100 µl** or greater.
f. Spin down tubes at **12,000 g** for **8 mins**. Discard flow-through.
g. Create the **Antibody Reduction Master Mix** based on the number of CODEX® antibody conjugates.

Antibody Reduction Master Mix								
Number of Antibodies	1	2	3	4	5	6	7	8
Reduction Solution 1 [µL]	6.6	13.2	19.8	26.4	33	39.6	46.2	52.8
Reduction Solution 2 [µL]	275	550	825	1100	1375	1650	1925	2200
Total [µL]	281.6	563.2	844.8	1126.4	1378	1689.6	1971.2	2252.8

h. Add **260 µl** of the **Antibody Reduction Master Mix** to the top of each filter unit. Vortex for 3 seconds.
i. Incubate for **30 mins.** Exceeding 30 mins will result in irreparable damage to antibodies.
j. Spin down tubes at **12,000 g** for **8 mins**. Discard flow-through.
k. Add **450 µl** of **Conjugation Solution** to the top of each column.
l. Spin down tubes at **12,000 g** for **8 mins**. Discard flow-through.

3. Prepare CODEX® Barcodes and Conjugate Antibody

a. Resuspend each **Barcode** in **10 µl** of Molecular Biology Grade **Water** by pipetting up and down.
b. Add **210 µl** of **Conjugation Solution** to each **suspended Barcode**. Mix by gentle pipetting. Set aside.
c. Add the **CODEX® Barcode Solution** to the top of each filter. Close the lid and vortex for 3 seconds.
d. Incubate for **2 hours** at **RT**

4. Purify and Collect Tagged Antibodies

a. Set aside **2-5 µl** of the purified solution for QC and troubleshooting.
b. Spin down tubes at **12,000 g** for **8 mins**. Discard flow-through.
c. Add **450 µl** of **Purification Solution** to the top of each column.
d. Spin down tubes at **12,000 g** for **8 mins**. Discard the flow-through.
e. **Repeat steps c and d** two more times for **a total of three** purifications.
f. After the **third centrifugation, discard** the flow-through solution.
g. The top of the column will contain the remaining purified solution.
h. For each antibody, **label a new tube** and lid that can hold filter units..
i. Add **100 µL** of **Antibody Storage Solution** to each filter unit column.
j. After it is labeled, **place** the new empty **tube upside-down** on top of the filter unit column.
k. **Invert the filter** for collection into the new collection tube.
l. Spin solution down at **3,000g for 2 mins**. The **final volume** in the tube should be **around 120 µl.**
m. Transfer the solution to a sterile, screw-top tube for storage at **4°C** for up to **1 year**.
n. Do not use these antibodies for tissue staining for at least 2 days; if used for staining sooner, you may experience high levels of background nuclear staining.

5. Quality Control of Conjugated Antibodies

a. **Dilute** conjugated antibodies and the unconjugated antibody (control) to a **final volume** of **13 µl** in Nuclease free water.
b. Add **5 µl** of NuPAGE™ LDS Sample Buffer to each sample.
c. Add **2 µl** case NuPAGE™ Sample Reducing Agent to each sample.
d. Denature at **95°C** in a dry bath for **5 mins.**
e. Prepare buffer by adding **25ml** of NuPAGE MOPS SDS Running Buffer and **475ml** of of NuPAGE MOPS SDS Running Buffer.
f. Prepare gel according to manufactures instructions.
g. Pour buffer into gel tank.
h. Add **5 µl** of a pre-stained protein standard 3.5-260 kDa to the gel.
i. Add **20 µl** of antibody to one well each.
j. Run the gel **at 200 V** for **30-40 mins** until completion.
k. Place in a container filled with ddH2O.
l. **Microwave** the gel until the **first bubbles form**.
m. Stain the gel with Novex SimplyBlue™ SafeStain.
n. **Microwave** the gel again until the first **bubbles form**.
o. Place the gel in a shaker for **10 mins**.
p. Wash the gel with ddH2O and leave it on the shaker until bands are visible.

Antibody:

- 1 µg of each conjugated antibody

Additional Materials

- NuPAGE™ LDS Sample Buffer (4X)
- NuPAGE™ Sample Reducing Agent (10X)
- NuPAGE™ 4-12% Bis-Tris Protein Gels
- Novex™ Sharp Pre-Stained Protein Standard - 3.5-260 kDa
- XCell SureLock™ Mini-Cell Electrophoresis System
- NuPAGE™ MOPS SDS Running Buffer (20X)
- Novex™ SimplyBlueTM SafeStain ddH2O
- 95°C dry bath
- Microwave
- Shaker