AKOYA BIOSCIENCES®
THE SPATIAL BIOLOGY COMPANY®

*We've rebranded some of our products. **CODEX**® is now **PhenoCycler**™.*

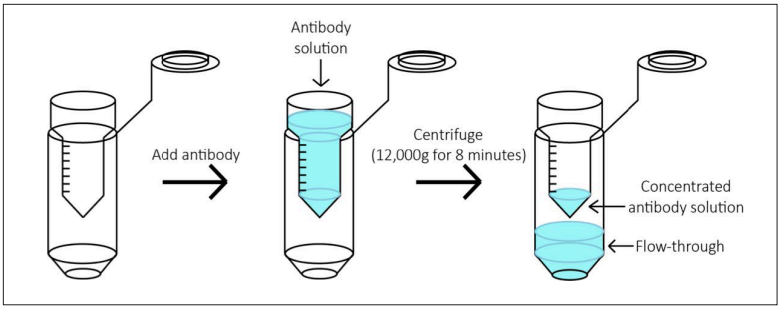
Conjugating PhenoCycler Tags on Antibodies of Choice

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 after 30-min incubation for step 4.1.5 :
- Conjugation Solution
- Barcodes

f. Retrieve the following reagents after 2-hour incubation for step 4.1.8 :
- 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
· PhenoCycler Conjugation Kit
- Reduction Solution 1
- Reduction Solution 2
- Filter Blocking Solution
- Conjugation Solution
- Purification Solution
- Antibody Storage Solution
- PhenoCycler Barcodes

Materials NOT Included in Kit
· Biologics:
- Purified antibody(s)

· Consumables:
- 50kDa MWCO filter
- 1.5 mL screw-top sterile tube(s)
- Nuclease-Free Molecular Biology Grade Water
- PBS
- 0.2 mL PCR tubes (for quality check, Section 4.2)
- Bucket of ice for antibodies
- Instrumentation:
- Centrifuge for 1.5 mL tubes
- NanoDrop™ spectrophotometer
- Vortex (Optional)

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
e. necessary to remove all liquid on top of the column. 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 PhenoCycler 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 2-3 seconds.
i. Incubate at RT 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 PhenoCycler Barcodes and Conjugate Antibody

a. a. Resuspend each **Barcode** in **10 µl** of Molecular Biology Grade **Water** by pipetting up and down.

b. b. Add **210 µL** of **Conjugation Solution** to each **suspended Barcode**. Mix by gentle pipetting. Set aside.

c. Add the **PhenoCycler 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. The filter will contain the conjugated antibody 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. Validating Custom-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 **10 mins**.

e. Prepare buffer by adding **40 mL** of NuPAGE MOPS SDS Running Buffer and 760 mL 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 ddH_2O.

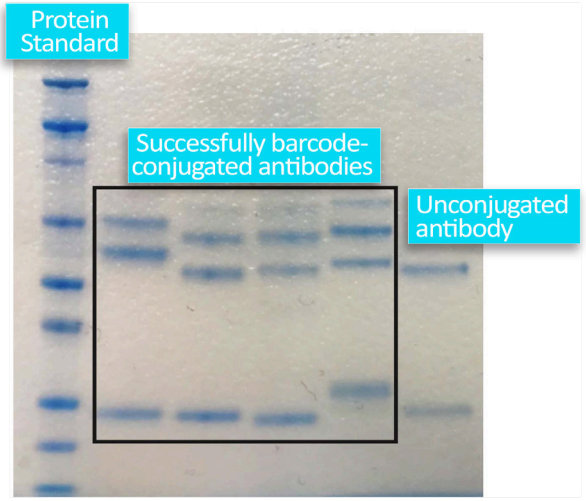
l. **Microwave** the gel until the **first bubbles form**.

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.



From left to right: the first column shows the protein standard, columns from the second to the fifth show bands of successfully barcode-conjugated antibodies.

The last column shows the heavy and light chain bands from a control, unconjugated antibody.

Antibody
- 5 µL of each conjugated antibody from section
- 4.1.9a.
- 1 µg (usually corresponding to 2 µL) of
- unconjugated antibody to be used as control.

Materials NOT Included in Kit
- Reagents:
- NuPAGE™ LDS Sample Buffer (4X)
- NuPAGE™ Sample Reducing Agent (10X)
- NuPAGE™ 4-12% Bis-Tris Protein Gels
- Novex™ Sharp Pre-Stained Protein Standard -3.5260
- kDa
- XCell SureLock™ Mini-Cell Electrophoresis System
- NuPAGE™ MOPS SDS Running Buffer (20X)
- Novex™ SimplyBlue™ SafeStain
- ddH2O
- Nuclease-free water
- Instrumentation:
- 95°C dry bath
- Microwave

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