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CODEX Oligo-labeled Antibody Conjugation V.2

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Human Cell Atlas Metho...

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We use this protocol and it's working

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Abstract

CODEX is a technology that uses oligo labeled antibodies, specialized fluorescent probes, and a companion instrument along-side a standard fluorescence microscope to create single-cell resolution fluorescence data across a multitude of parameters within spatial context in a single tissue. CODEX was developed by Yury Goltsev and Nikolay Samusik in the laboratory of Garry Nolan at Stanford. The technology is being commercialized by **Akoya Biosciences**.

Guidelines

Purified antibody stock can not be stored with any carrier protein or glycerol. Request the vendor to provide purified antibody in a BSA-free form.

A pre-purification process must be performed if a BSA-free antibody stock cannot be found.

Measure concentration of purified antibody with nanodrop before conjugation procedure. Concentrations on the tube label are not always accurate.

Materials

	PRODUCT	PROVIDER	CATALOG NUMBER
	50KDa MWCO filter	Millipore	UFC505096
	Antibody Reduction Solution 1	Akoya	RGT2004EA
	Antibody Reduction Solution 2	Akoya	RGT2004EA
	Antibody Conjugation Solution	Akoya	RGT2004EA
	Filter Blocking Solution	Akoya	RGT2004EA
	CODEX Antibody Tags	Akoya	RGT2004EA
	Antibody Purification Solution	Akoya	RGT2004EA
	Antibody Storage Solution	Akoya	RGT2004EA
	Nanodrop 2000c	ThermoFisher	ND-2000c
	Eppendorf microcentrifuge	Eppendorf	5418

Troubleshooting

Antibody Disulfide Reduction Reaction

1 Retrieve one MWCO filter column for each antibody to be conjugated. Block nonspecific antibody binding to the MWCO filter columns by adding 500ul Filter Blocking Solution to the top of each column and spinning down at 12,000g for 2 minutes.

12000 x g
00:02:00
Room temperature

2 Aspirate all liquid from the top of each MWCO filter column and discard flow-through in the collection tube. Exercise caution and do not scrape and damage the filter with the aspirator tip.

3 Measure concentration of stock purified antibody by nanodrop with pre-set IgG settings after blanking against the buffer they are suspended in, usually PBS. Calculate the volume needed for 50ug of antibody based on the nanodrop measurement.

4 Add 50ug of antibody to the top of each MWCO filter column and spin down at 12,000g for 8 minutes. The resulting solution should be 50ug of antibody concentrated into approximately 25ul.

12000 x g
00:08:00
Room temperature

5 Discard flow-through.

6 Prepare ***Antibody Reduction Master Mix*** by combining ***Antibody Reduction Solution 1*** with ***Antibody Reduction Solution 2***.

20ul of ***Antibody Reduction Solution 1*** is needed for every 3 antibodies to conjugate.

20ul of ***Antibody Reduction Solution 1*** is mixed with 825ul of ***Antibody Reduction Solution 2***.

7 Add 260ul ***Antibody Reduction Master Mix*** to the top of each MWCO filter column. Gently pipet up and down to mix reagent with antibody. Incubate at RT for 30 minutes.

00:30:00
Room temperature

8 After 30 minutes spin down the MWCO filter columns at 12,000g for 8 minutes.

12000 x g
00:08:00
Room temperature

9 Discard flow-through.

Antibody Conjugation Reaction

10 Add 450ul ***Antibody Conjugation Solution*** to the top of each MWCO filter column and spin down at 12,000g for 8 minutes. During this spin, prepare the antibody tags.

12000 x g
00:08:00
Room temperature

11 The antibody tag preparation is time sensitive and must be done immediately prior to use. The antibody tag aliquots are to be used once for every 50ug antibody conjugation reaction.

12 Pipet 220ul ***Antibody Conjugation Solution*** into each antibody tag aliquot. Ensure that the aliquot is dissolved with gentle pipetting.

13 After the spin in step 10 is completed, discard flow-through and add the dissolved antibody tag solutions from step 12 into each corresponding MWCO filter column. Pipet up and down gently to mix the reagents.

14 Close the MWCO filter column lids and incubate the conjugation reaction at RT for 2 hours.

02:00:00
Room temperature

15 After 2 hours, spin down the MWCO filter columns at 12,000g for 8 minutes.

12000 x g
00:08:00
Room temperature

16 Discard flow-through.

Antibody Purification and Storage

17 Add 450ul ***Antibody Purification Solution*** to each MWCO filter column and spin down at 12,000g for 8 minutes.

12000 x g
00:08:00
Room temperature

Discard flow-through.

18 Add another 450ul ***Antibody Purification Solution*** to each MWCO filter column and spin down at 12,000g for 8 minutes.

12000 x g
00:08:00
Room temperature

Discard flow-through.

19 Add another 450ul ***Antibody Purification Solution*** to each MWCO filter column and spin down at 12,000g for 8 minutes.

12000 x g
00:08:00
Room temperature

Discard flow-through.

20 Add 100ul ***Antibody Storage Solution*** to the top of each MWCO filter column. Gently pipet up and down 10 or more times and wash the sides of the filters in the column. 50ug antibody should be dissolved in 100ul ***Antibody Storage Solution***. If the conjugation scale is larger than 50ug, add more ***Antibody Storage Solution*** according to this ratio.

21 Invert the filters into a fresh collection tube. Spin down at 3000g for 2 minutes. KEEP THE COLLECTED SOLUTION.

3000 x g
00:02:00

22 Pipet the conjugated antibody solutions into sterile screw-top tubes and store for one year at 4 °C .