

Autofluorescence Quenching Protocol for CODEX®

Featured Customers: Derek Oldridge, M.D. Ph.D. and Jonathan Belman M.D. Ph.D., Department of Pathology and Laboratory Medicine, University of Pennsylvania. Laboratory of John Wherry Ph.D., Department of Systems Pharmacology and Translational Therapeutics and Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, PA, USA.

Additional Source: Du et al. 2019. Nature Protocols 14: 2900-2930.

EQUIPMENT

This protocol requires two broad-spectrum LED light sources at 20'000 to 25'000 Lux. We recommend purchasing two A4-sized, Aibecy A4 Ultra Bright 25'000 Lux LED Light Box-Tracing Pads as available here: (<https://www.amazon.com/Aibecy-Stepless-Sketching-Animation-Stencilling/dp/B07R66KRXV>). Excessive heat damages tissues and LEDs are thus preferable.

Please note that this photobleaching protocol has been tested at two stages in the CODEX workflow as below. Both methods reduced background fluorescence without an appreciable decrease in biomarker signal intensity.

1. At the end of the CODEX protocol immediately before placement into the storage buffer (just before step 5.6.8 in the Rev B.0 protocol).
2. Immediately after antigen retrieval (step 5.4.3 in the Rev B.0 protocol).

NOTE: *Customer Demonstrated CODEX Protocols are not validated and tested by Akoya's R&D team and a performance or support guarantee is not provided for these protocols. Please contact your local account manager for further clarification.*

PROTOCOL

1. Prepare the Bleaching solution in a 50mL centrifuge tube by combining:
 - 25mL of 1x PBS
 - 4.5mL of 30% (wt/vol) H₂O₂ (Sigma-Aldrich #216763)
 - 0.8mL of 1M NaOHFinal working solution: 4.5% (w/v) H₂O₂ and 20mM NaOH in PBS. Adjust volumes of reagents as needed for your experiment.
2. Submerge the coverslip in the Bleaching solution using the 6-well plate format used elsewhere in the Akoya protocol. Sandwich the 6-well plate between the two LED lamps for 45 minutes at room temperature.

Please note: The distance between the 6-well plate and the light sources may be adjusted on a case by case basis but we recommend placing the plate directly on top of the bottom light, while placing to top light directly overhead.
3. After 45 minutes, replace the Bleaching solution or move the coverslip into a new well with fresh Bleaching solution.
4. Repeat photobleaching for another 45 minutes at Room Temperature.

Please note: The duration of photobleaching can be varied as different tissue types may require shorter or longer exposures. The protocol described here was successfully tested in normal human placenta, testis, brain, liver, heart, spleen, kidney and lung (clinical FFPE cores arrayed on a TMA for all). Overall we observed good background reduction that was reproducible across multiple experiments.
5. Wash the tissues four times in 1x PBS for 3-5 minutes per wash.
6. Place the tissue in storage buffer for equilibration.
7. Initiate CODEX run when ready.

To learn more about CODEX technology, visit akoyabio.com/codex

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