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We use this protocol in our group and it is working.

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CODEX - Poly-L-Lysine Cover-Slip Preparation V.2

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ABSTRACT

Poly-Lysine Coverslip Preparation

This section describes the process of creating Poly-lysine-coated coverslips that are used for the tissue sections in the CODEX® experiment workflow.

GUIDELINES

- Managers and supervisors - are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
- Laboratory personnel - are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs.

MATERIALS

MATERIALS

WypAll™ L30 Multipurpose Wipers **Thermo Fisher Catalog #19168203**

Parafilm Wrap PM996, 4 in. Wide, 125 ft./Roll **Thermo Fisher Catalog #CP0672040**

Dumont Forceps (Cover Slip Forceps) **Fine Science Tools Catalog #11251-3**

EMS Glass Cover Slips 22mm x 22mm **Electron Microscopy Sciences Catalog #72204-01**

Poly-L-lysine 0.1% (w / v) **Sigma Aldrich Catalog #P8920**

SAFETY WARNINGS

- Use physical safety precautions when working with sharps (disposable blades, coverslips, etc).

BEFORE START INSTRUCTIONS

- Be sure to gather all the appropriate materials listed in this protocol before starting.
- You will need a sanitized or acid-washed beaker for this procedure.

1 Remove the EMS cover-slips from box.

1m

CODEX requires use of these specific cover-slips for their diagnostic process.

2 Gently place the desired amount of cover-slips that you wish to coat in solution at the bottom of a sterilized glass beaker.

1m

3 Slowly swirl the beaker to spread the cover-slips around the base.

30s

4 Add 7 mL of poly-lysine solution above the cover-slips to ensure that all are fully covered.

2m

7 mL Poly-Lysine

5 Cover the beaker with plastic wrap or parafilm, and secure tightly with a rubber band to prevent evaporation.

2m

6 Leave cover-slips in poly-lysine solution for a **minimum** of 12 hours and up to one week **at room temperature**.

12h

12:00:00 or up to one week.

7 After waiting the required incubation period, carefully remove the rubber band and parafilm from around the neck of the beaker.

2m

8 Gradually pour the remaining poly-lysine solution into the proper waste container.

2m

9 Fill the same beaker containing the cover-slips to half volume with double-distilled water(ddH₂O) or purified water.

2m

10 Swirl the contents to mix the solution.

1m

11 Let the beaker and cover-slips sit for 30 seconds.

30s

To prevent removal of poly-lysine, do not soak in water for >1 minute during each washing step.

12	Slowly pour off the water into the sink. This completes Wash #1	30s
13	⇒ go to step #9 and repeat steps 9-12 (Wash #2)	1m 30s
14	⇒ go to step #9 and repeat steps 9-12 (Wash #3)	1m 30s
15	⇒ go to step #9 and repeat steps 9-12 (Wash #4)	1m 30s
16	⇒ go to step #9 and repeat steps 9-12 (Wash #5)	1m 30s
17	⇒ go to step #9 and repeat steps 9-12 (Wash #6)	1m 30s
18	⇒ go to step #9 and repeat steps 9-12 (Wash #7)	1m 30s
19	After completing the 7 washes, place 2 Wypall towels or lint-free paper towels on the bench top.	30s

- 20 Remove the cover-slips from the water, placing them on top of the first set of towels. Ensure the cover-slips are not overlapping to allow proper drying.

- 21 Using the specialty forceps indicated for use by CODEX, flip over each cover-slip onto the second clean towel to dry the reverse side.

Specialty forceps indicated in the materials section.

- 22 Leave the cover-slips on the Wypall towel to dry.

- 23 When the cover-slips are dry, the Poly-Lysine-coated cover-slips can be stored in a sanitized petri dish or similarly covered container for up to 2 (two) months.

Sanitized Petri Dish containing the finished Poly-L-Lysine coated cover-slips for CODEX processing.