

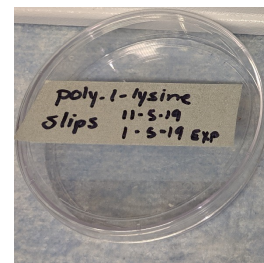
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Version 2

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

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Protocol status: Working

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

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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 #11251-33**
- ✂ EMS Glass Cover Slips 22mm x 22mm **Electron Microscopy Sciences Catalog #72204-01**
- ✂ Poly-L-lysine 0.1% (w / v) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P8920**

Safety warnings

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

Before start

- 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.





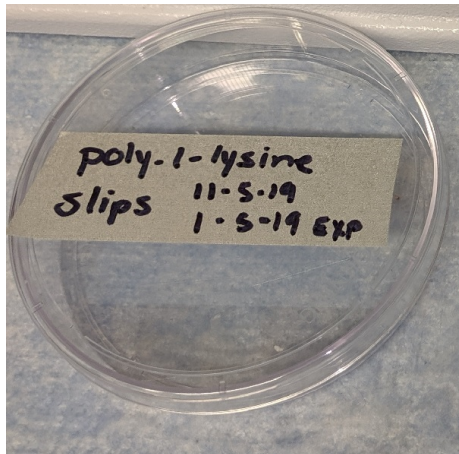
- | | | |
|----|---|--------|
| 7 | After waiting the required incubation period, carefully remove the rubber band and parafilm from around the neck of the beaker. | 2m |
| 8 | Gradually pour the remaining poly-lysine solution into the proper waste container. | 2m |
| 9 | Fill the same beaker containing the cover-slips to half volume with double-distilled water(ddH ₂ O) 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 <u>step #9</u> and repeat steps 9-12 (Wash #2) | 1m 30s |
| 14 | go to <u>step #9</u> and repeat steps 9-12 (Wash #3) | 1m 30s |
| 15 | go to <u>step #9</u> and repeat steps 9-12 (Wash #4) | 1m 30s |
| 16 | go to <u>step #9</u> and repeat steps 9-12 (Wash #5) | 1m 30s |
| 17 | go to <u>step #9</u> and repeat steps 9-12 (Wash #6) | 1m 30s |
| 18 | go to <u>step #9</u> 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. 2m
- 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.

