CODEX 實驗流程注意事項

一、 組織切片

1. 請使用原廠 coverslips 玻片(22 mm x 22 mm)進行實驗。
2. Coverslips 須先用 0.1% poly-L-lysine solution 處理後方能使用，本組提供已製備好之 coverslips 供研究人員購買使用。
   (若須自行製備請參閱附件一實驗流程)
3. 0.1% poly-L-lysine coated coverslips 須於 2 個月內使用完畢。
4. 組織切片之厚度建議為 5-10 μm，並保持組織平面完整性，不可有皺褶或破碎狀態。
5. 冷凍切片組織可保存於-80℃冰箱 6 個月；石蠟包埋切片可保存於 4℃冰箱 6 個月。
6. 組織切片流程及注意事項請參閱附件二實驗流程及 Tissue Processing – Best Practices 文件。

二、 抗體標記 (實驗試劑及器材需自行準備)

1. 實驗試劑：CODEX staining kit、16% Paraformaldehyde (研資組供售)、1x PBS、Acetone、4℃ methanol。
2. 實驗器材：玻片載物盒 2 個、專用鑷子、乾燥劑、6-well plate 10 個、200 ml 燒杯 2 個、1000 µl pipette/tips、200 µl pipette/tips、20 µl pipette/tips。
3. 抗體標記實驗流程依切片種類分別請參閱 one sheet protocol v3 及 Tissue Staining and Reporter Plate preparation 文件。
4. 抗體標記完成之組織切片須浸泡於 Storage Buffer 保存於 4℃冰箱，並須在 5 日內完成螢光染色實驗。

三、 螢光染色

1. 實驗試劑：Nuclease Free Water、10X CODEX Buffer (研資組供售)、Assay Reagent (研資組供售)、Nuclear Stain (研資組供售)、CODEX reporters 螢光試劑。
2. 實驗器材：96-well plate/foil seal 1 組 (研資組供售)、專用鑷子、1000 µl pipette/tips、200 µl pipette/tips、20 µl pipette/tips。
3. 螢光染色實驗流程依切片種類分別請參閱 Tissue Staining and Reporter Plate

preparation 文件。

4. Assay Reagent、Nuclear Stain 及 CODEX reporters 使用後須保存於 4℃冰箱，不可再回凍於-20℃冰箱。
5. 螢光染色完成之 96-well plate 可保存於 4℃冰箱，並須在 2 個禮拜內完成儀器上機。

四、 儀器上機

1. 實驗試劑：1X CODEX Buffer (研資組供售)、Nuclear Stain (研資組供售)、DMSO (研資組供售)、ddH_2O。
2. 實驗器材：專用鑷子、1000 µl pipette/tips、2 µl pipette/tips。
3. 儀器上機流程請參閱 CODEX Run_Keyence 800 Setup 文件。
4. 本儀器全程由技術員操作，使用者不可擅自操作儀器。
5. 儀器運作、資料處理及轉檔期間，不可隨意碰觸儀器及電腦，以免影響儀器正常運作。
6. 儀器使用完畢後須保持儀器、實驗桌及周遭環境整潔，並將個人物品與廢棄物帶走，不可隨意丟棄於實驗桌上。
7. 研究資料請自行準備已格式化之隨身硬碟，經技術員確認許可後方能存取；本組代為保存檔案期限為 6 個月，超過期限之檔案，經技術員與使用者聯繫且同意刪除後再執行刪除作業。

附件一 Coverslips 製備

Guidelines

Preparation and storage period:

- Please work with the recommended coverslips from Akoya Biosciences. Other brands of coverslips may be too fragile or inaccurately sized and can easily break during CODEX® experiments.
- The preparation of poly-L-lysine coated coverslips requires a minimum incubation of 12 hours. It is recommended that coverslips are treated with poly-L-lysine at least 2 days prior to tissue sectioning.
- The coverslips can be incubated in poly-L-lysine for a maximum of 1 week.
- Poly-L-lysine-coated coverslips must be used within 2 months.

Incubating Coverslips

a. Remove the coverslips from the box.
b. Gently place coverslips at the bottom of the glass beaker.
c. Slowly swirl the beaker to spread the stacks of coverslips across the bottom of the beaker.
d. Add enough poly-L-lysine solution to the beaker to ensure that all coverslips are fully covered with solution. If coating a whole box of coverslips, typically 70 mL of poly-L-lysine solution will be sufficient for covering all coverslips.
e. Mix the solution and coverslips by rotating the beaker at a 45° angle for 1 minute, ensuring that all coverslips are fully immersed in the solution. Use a pipette tip to remove any air bubbles and coverslips that are stuck together.

NOTE	Coverslips should be dispersed to maximize the surface area of each coverslip exposed to the solution. Minimize the number of coverslips sticking and overlapping with one another.

f. Cover the beaker with parafilm to prevent evaporation.
g. Leave coverslips in the poly-L-lysine solution for a minimum of 12 hours and up to one week at room temperature (RT).

INCUBATE	Minimum 12-hour incubation at RT

STOPPING POINT	Leave coverslips in poly-L-lysine solution for a minimum of 12 hours and up to one week at RT

Washing and storing coverslips

Guidelines

Coverslips

- To prevent removal of poly-L-lysine, do not soak in water for >1 minute during each washing step.
- Dried poly-L-lysine coated coverslips can be stored for up to 2 months at RT.

Reagents

- Milli-Q® ultrapure water (Type 1) or double-distilled H_2O should be used. Deionized H_2O is not recommended.

Pre-Experiment Preparation

Materials NOT included in Kit

- Lint-free drying surface or paper towels
- ddH_2O
- Petri dish or similar container

Washing Coverslips

a. Slowly pour the poly-L-lysine solution into the proper waste disposal container.
b. Fill the beaker containing the coverslips to half volume with ddH_2O.
c. Swirl the contents to mix the solution.
d. Let the beaker and coverslips sit for 30 seconds.
e. Slowly pour off the water into the sink.
f. Repeat steps b – e, for a total of 5-7 washes.
g. Place two sets of paper towels on the benchtop.
h. Remove the coverslips from the beaker and place them in a single layer on top of the drying surface or a first set of paper towels. Ensure coverslips do not stick together as they will not properly dry.

NOTE Coverslips can be removed from the beaker in batches.

i. Let the coverslips dry for several hours.
j. If needed for complete drying, invert each coverslip. Dry the reverse side on the drying surface or a second set of paper towels.
k. Leave the coverslips on the drying surface or paper towels to dry overnight.
l. When the coverslips are completely dry, the poly-L-lysine-coated coverslips can be stored in a petri dish or similar container.

STOPPING POINT Place poly-L-lysine coated coverslips in a petri dish for storage for up to 2 months.

附件二 組織切片

3.2 Fresh-Frozen Tissue Sectioning

Fresh-frozen tissue sections are mounted directly onto poly-L-lysine-coated coverslips. Appropriate preparation and storage of tissue sections are critical to ensure sample integrity. The instructions provided in this manual are specific to the CODEX® workflow, and they are not intended to be a comprehensive guide for tissue processing. Further guidance on tissue processing of fresh-frozen samples can be found on our website, "Guidelines: Tissue processing – Best practices" at akoyabio.com.

Guidelines

Tissue Sections

- Tissue sections adhered to poly-L-lysine-coated coverslips can be stored at -80°C for up to 6 months before staining.
- It is critical that tissue thickness does not exceed 10 µm as this can affect the autofocusing capabilities of the microscope.
- For best results, tissue sections should be completely adhered to the coverslip without folds or tears.
- To ensure that tissue sections are not damaged, it is critical that the tissue coverslips are not stacked on top of one another.

Pre-Experiment Preparation

Materials Included in Kit

- CODEX® Coverslip Storage box, 7000013

Materials NOT Included in Kit

- Poly-L-Lysine-coated coverslips prepared in section 3.1.
- Fresh-frozen tissue block of interest
- Compressed/canned air duster
- Dry ice
- Polystyrene container for Dry ice
- Cryostat for tissue sectioning
- Blade for tissue sectioning (we recommend 63069-LP Low Profile Microtome Feather® Blade by Electron Microscopy Sciences)

Prepare Cryostat Chamber

Standard cryostats with temperature control are recommended for tissue sectioning. Most tissues are sectioned in temperatures ranging from -15°C to -25°C. The exact temperature is unique to each tissue type and should be determined according to standard sectioning procedures.

Fresh-Frozen Tissues - Sectioning Instructions

a. Set the cryostat chamber to tissue-specific temperature range.
b. Place the CODEX® Coverslip Storage box in the cryostat chamber to equilibrate to the cryostat temperature.
c. Once the cryostat reaches the programmed temperature, transfer the tissue from the -80°C freezer to the cryostat using a container filled with dry ice.
d. Use compressed air to remove dust and lint from the coverslips before use.
e. Place the prepared poly-L-lysine-coated coverslips in the cryostat chamber to equilibrate temperature for 20-30 seconds.
f. Section the tissue at a thickness of 5-10 µm.

CRITICAL	Do not exceed 10 µm as this will affect the autofocusing capabilities of the microscope. Avoid folds and tears in the tissue, as these artifacts will affect image quality and data analysis.

g. Gently place the tissue section in the center of the coverslip.
h. Adhere the tissue section to the coverslip by placing a gloved finger underneath the coverslip for 1-2 seconds.

CRITICAL	Do not keep your finger on the coverslip for longer than the minimum time necessary to melt the OCT.

NOTE	The directed heat transfer should melt the OCT, thereby ensuring tissue adherence. Chemical fixation of the tissue will take place during the staining protocol.

i. Place the mounted coverslip in a single slot of the CODEX® Coverslip Storage box.
j. Repeat steps f - i for each tissue section.
k. Once complete, cover the CODEX® Coverslip Storage box with the lid.
l. Place the box of mounted coverslips on dry ice for transport to a -80°C freezer.

STOPPING POINT	If prepared and stored properly, samples can be stored at -80°C for up to six months. Limit exposure to changes in temperature and keep storage box upright and secure as to minimize movement of coverslips.

NOTE	Tissue processing and sectioning are critical steps and need to be performed by trained users. Resources for tissue processing best practice procedures can be found on our website, "Guidelines: Tissue processing – Best practices" at akoyabio.com