Jan 07, 2026 Version 1

Xenium in situ Gene Expression for FFPE – IU KPMP TIS V.1

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvwn6nwvmk/v1

Ricardo Melo Ferreira[1], Ying-Hua Cheng[1], Michael Eadon[1]

[1]Indiana University School of Medicine

KPMP
Tech. support email: **info@kpmp.org**

Ricardo Melo Ferreira
Indiana University

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwn6nwvmk/v1

Protocol Citation: Ricardo Melo Ferreira, Ying-Hua Cheng, Michael Eadon 2026. Xenium in situ Gene Expression for FFPE – IU KPMP TIS. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwn6nwvmk/v1Version created by **Ricardo Melo Ferreira**

Protocol status: Working
We use this protocol and it's working

Created: December 30, 2025

Last Modified: January 07, 2026

Protocol Integer ID: 236016

Keywords: xenium in situ gene expression, cg000749-xeniuminsitu-geneexp-cellsegmentation-user-guide-revb, cg000580-demonstrated-protocol-xenium-ffpe-deparaffinization-decrosslinking-reva-2, xenium, kidney precision medicine project, situ gene expression, indiana university tissue interrogation site

Abstract

This is the protocol used by the Indiana University Tissue Interrogation Site for the Kidney Precision Medicine Project. Since Xenium is a commercial assay, this document is strongly based on the protocols published by 10X, with changes made when necessary based on our workflow. The 10X guides used for this protocol are CG000578_Demonstrated_Protocol_XeniumInSituProtocolsFFPE_TissuePreparationGuide_RevA, CG000580_Demonstrated_Protocol_Xenium_FFPE_Deparaffinization_Decrosslinking_RevA_2, CG000749_XeniumInSitu_GeneExp_CellSegmentation_User_Guide_RevB, and CG000613_Demonstrated_Protocol_Xenium_H_EStaining_RevB

Before start

Adding and removing reagents from the xenium cassette should always be always done from the corner of the well, ensuring uniform coverage without introducing bubbles or disrupting the tissue.

Post Amplification Wash

1 Wash with 500 µl TE Buffer, incubate 1 min at RT, then remove all buffer, twice

2 Wash with 500 µl TE Buffer. Place new lid and keep it room temperature during buffer preparation. Removal of buffer will be on a posterior step.

Cell Segmentation Staining

3 Prepare 100% and 70% Ethanol mixes

4 Prepare Diluted Xenium Block 6 Stain Buffer (for two slides). Add in order 990 µl of Diluted Buffer.

5 Aspirate all TE buffer from cassette. Save lid.

6 Four ethanol washes with two minutes incubation at room temperature and 1,000 µl each: 70%,100%, 100%, 70%.

7 Do not let tissue dry. Immediately add 1,000 µl PBS-T. Incubate for 1 min at room temperature. Remove all buffer.

8 Add 500 µl of Diluted Xenium Block 6 Stain Buffer. Apply saved lid and incubate 1 h at room temperature. Keep remaining buffer for later steps.

9 During incubation, prepare Xenium Multi-Tissue Stain Mix. Add 220 µl of Diluted Block and Stain Buffer to the Xenium Multi-Tissue Stain Mix tube, pipette-mix 15X (175 µl setting) and centrifuge briefly. Incubate 30 min at room temperature then centrifuge for 10 min at 14,000 rcf at 4°C and keep on ice.

10 Remove Diluted Xenium Block 6 Stain Buffer from cassette. Save the lid. Place cassette insert using forceps.

11 Pipette 100 µl Stain Mix under the insert via cut-out. Seal with Slide Seal. Incubate overnight (16–24 h) at 4°C.

12 On next day, thaw Xenium Stain Enhancer 10 min at room temperature. Centrifuge briefly and confirm powder at bottom. Add 1,100 µl of PBS and pipette-mix 5X. Centrifuge for 5 seconds.

13 Remove Slide Seal from cassette. Add 200 µl PBS-T into insert cut-out to float insert. Remove insert with forceps and discard it. Remove residual Stain Mix.

14 Wash with 500 µl of PBS, incubate for 1 min at room temperature, remove all buffer, three times.

15 Add 500 µl of Stain Enhancer. Apply saved lid and incubate 20 min at room temperature.

16 Remove Stain enhancer and discard lids.

17 Wash with 500 µl of PBS-T, incubate for 1 min at room temperature, remove all PBS-T, twice. Add 500 µl PBS-T.

Autofluorescence Quenching

18 Prepare 100% and 70% Ethanol mix.

19 Prepare Reducing Agent B. For one (two) slides, add 544.5 (1,089.0) µl of PBS and 5.5 (11) µl of Reducing Agent B.

20 Prepare Autofluorescence Solution. For one (two) slides, add 544.5 (1,089.0) µl of 100% ethanol and 5.5 (11) µl of Autofluorescence Mix.

21 Remove PBS-T from cassette. Add 500 µl of Diluted Reducing Agent B. Apply new Cassette Lid and incubate 10 min at room temperature. Remove Agent B and save lid.

22 Three ethanol washes with 1 minute incubation at room temperature and 1,000 µl each: 70%, 100%, 100%.

23 Add 500 µl of Autofluorescence Solution. Apply Cassette Lid and incubate for 10 min at room temperature in the dark.

24 Three 100% ethanol washes with 2 minute incubation at room temperature and 1,000 µl each. Don’t need to be performed in the dark

25 Place cassette without lid on thermocycler and dry for 5 minutes on 37°C adaptor to dry. Don’t close cycler lid.

26 Rehydrate with 1,000 µl of PBS. Incubate for 1 min at room temperature in the dark. Remove PBS.

27 Add 1,000 µl of PBS-T. Incubate for 2 min at room temperature in the dark.

28 Incubate overnight (16h -24h) at 4°C in the dark with Cassette Lid.

Nuclei staining

29 Thaw Xenium Nuclei Staining Buffer.

30 Remove PBS-T from cassette. Add 500 µl Xenium Nuclei Staining Buffer, incubate 1 min at room temperature in the dark. Remove all PBS-T.

31 Wash with 1000 µl of PBS-T, incubate for 1 min at room temperature in the dark, remove all PBS-T, three times.

32 Add 1,000 µl PBS-T.

33 Proceed to Xenium Analyzer run or store slides.

34 Short-term storage ≤1 week at 4°C in dark with Cassette Lid or slide seal on nuclease-free, microbe-free conditions.

Quencher Removal

35 Thaw Xenium Nuclei Staining Buffer.

36 Prepare Quencher Removal. Add 17.4 mg of Sodium hydrosulfite to 10.0 ml of Milli-Q Water on a fume hood. Vortex on a tightly capped tube.

37 Remove residual PBS-T.

38 Remove slide from Xenium Cassette.

39 Immerse slide in slide mailer with 10 ml of Quencher Removal. Incubate for 10 min at room temperature (ensure tissue fully submerged).

40 Transfer to a new mailer with 10 ml Milli-Q Water, incubate for 1 min at room temperature, three times. Use new mailer with fresh Milli-Q Water each time.

41 Proceed directly to H&E staining, or store temporarily in 1x PBS at 4 °C (≤2 days).

H&E staining

42 Filter Hematoxylin and Eosin solutions before use.

43 Prepare 16 coplin jars filled to capacity: Hematoxylin x1; Bluing x1; 70% EtOH x1; 95% EtOH x3; Eosin x1; 100% EtOH x2; Xylene x2; Milli-Q Water x5.

44 Gently immerse the slides in the following jars and incubate at room temperature for the respective times:

45 Milli-Q Water 1: 2 min.

46 Hematoxylin: 20 min.

47 Milli-Q Water 2: 1 min.

48 Milli-Q Water 3: 1 min.

49 Milli-Q Water 4: 1 min.

50 Bluing solution: 1 min.

51 Milli-Q Water 5: 1 min.

52 70% EtOH: 3 min.

53 95% EtOH 1: 3 min.

54 Eosin: 2 min.

55 95% EtOH 2: 30 s.

56 95% EtOH 3: 30 s.

57 100% EtOH 1: 30 s.

58 100% EtOH 2: 30 s.

59 Xylene 1: 3 min (keep jar covered to limit evaporation).

60 Xylene 2: 3 min (keep jar covered to limit evaporation).

Imaging

61 Add 150–200 µl mounting medium, apply coverslip at an angle to avoid bubbles, allow media to spread. Air-dry 30 min at room temperature.

62 Store up to 3 days at 4 °C before imaging; after imaging, store at RT in the dark.

63 Slides are imaged on a Keyence BZ-X810 or an Evos M7000. The Keyence is equipped with a Nikon PlanFluor 10X and a Nikon Plan Apo 20X, while the Evos has Olympus LUCPlanFLN 20X and 40X objectives.

64 Mosaics are stitched by each microscope software or with ImageJ.