USER GUIDE

Glysite™ Explorer *in situ* PLA Glycan Detection Kit

Cat. No. **GEK-1000**

Glysite™ Explorer Lectins

Cat. No. **GEK-1029, GEK-1049, GEK-1119, GEK-1149, GEK-1159, GEK-1249, GEK-1269, GEK-1309, GEK-1359, GEK-1399**

Description

The Glysite™ Explorer *in situ* PLA Glycan Detection Kit is a fully integrated tool for the spatial detection of glycans proximal to a protein of interest in FFPE tissues, FFPE cell pellets, and fixed cells. This system integrates a curated panel of Vector's Glysite™ Explorer Lectins with Navinci's proprietary *in situ* Proximity Ligation Assay (isPLA). Researchers can flexibly select specific Glysite™ Explorer Lectins covering the major categories of glycan types to pair with a primary antibody targeting their protein of interest. This provides a simple approach to understand glycan-protein proximity with a spatial context.

Storage

Store reagents in original bottles at 4°C and -20°C accordingly.

Kit Components

Glysite™ Explorer *in situ* PLA Glycan Detection Kit, Box 1 (store at 4°C, do not freeze)

Product Name	Volume
BLOXALL® Blocking Solution	5 mL
Blocking Buffer 1	5 mL
Blocking Buffer 2A	5 mL
Blocking Buffer 2B (25x)	0.2 mL
Protein Diluent	15 mL
Lectin Probe (50x)	0.1 mL
Mouse Probe (50x)	0.1 mL
Rabbit Probe (50x)	0.1 mL
ImmPRESS® HRP Reagent (400x)	12.5 µL
ImmPRESS® HRP Diluent	5 mL
ImmPACT® DAB Reagent (33x)	0.153 mL
ImmPACT® DAB Diluent	5 mL
Hematoxylin QS	5 mL

Glysite™ Explorer *in situ* PLA Glycan Detection Kit, Box 2 (store at -20°C)

Product Name	Volume
Buffer 1 (5x)	1 mL
Enzyme 1 (40x)	0.125 mL
Buffer 2 (5x)	1 mL
Enzyme 2 (40x)	0.125 mL

Glysite™ Explorer Lectins (store at 4°C, do not freeze)

Please note: Lectin(s) are sold separately from the Glysite™ Explorer *in situ* PLA Glycan Detection Kit (GEK-1000) and at least one lectin from the list below is required to perform the assay.

Cat. No.	Product Name	Specificity*	Volume
GEK-1029	WGA Lectin (50x)	Terminal GlcNAcβ, Terminal GlcNAcα, Terminal *N*-acetyl-containing glycans	0.1 mL
GEK-1049	LCA Lectin (50x)	α1-6 Fucose	0.1 mL

Cat. No.	Product Name	Specificity*	Volume
GEK-1119	PHA-L Lectin (50x)	β1-6 Branched *N*-glycans, Binds tri- and tetraantennary	0.1 mL
GEK-1149	ECL Lectin (50x)	Terminal type 2 LacNAc, Terminal type 2 LacdiNAc	0.1 mL
GEK-1159	Jacalin Lectin (50x)	Core 1 and 3 *O*-glycans, 3-substituted GalNAcα	0.1 mL
GEK-1249	GNL Lectin (50x)	Terminal α1-3 or α1-6 mannose	0.1 mL
GEK-1269	MAL II Lectin (50x)	α2-3-Sialylated Galβ1-3GalNAc in *O*-glycans, 3' sulfated Galβ	0.1 mL
GEK-1309	SNA Lectin (50x)	α2-6 sialylated LacNAc, α2-6 sialylated LacdiNAc	0.1 mL
GEK-1359	WFA Lectin (50x)	Terminal GalNAcβ, Terminal GalNAcα, Terminal multiantennary LacNAc	0.1 mL
GEK-1399	AAL Lectin (50x)	α-Fucose	0.1 mL

**Bohar D, et al. 2022. A Useful Guide to Lectin Binding: Machine-Learning Directed Annotation of 57 Unique Lectin Specificities. ACS Chemical Biology.*

Additional Reagents & Equipment, Not Provided:

- Reagents to deparaffinize and rehydrate cells and tissue sections
- Antigen unmasking solution
- ImmEdge® Hydrophobic Barrier PAP Pen
- Humidified slide incubator with temperature control
- Mouse or Rabbit primary antibody
- 1X Tris-Buffered Saline (TBS) – contains 0.05 M Tris, 0.15 M sodium chloride, pH 7.5±0.1
- 1X Tris-Buffered Saline supplemented with 0.05% Tween-20 (TBST)
- Dehydration reagents
- Mounting medium
- Brightfield microscope

Recommended Experiments before using the Glysite™ Explorer *in situ* PLA Glycan Detection Kit:

- Perform standard Immunohistochemistry (IHC), Immunofluorescence (IF) or other assays to characterize expression/abundance of protein of interest and ensure the primary antibody is at the optimal concentration and shows correct specificity with great signal-to-noise ratios.
- Perform standard Immunohistochemistry (IHC), Immunofluorescence (IF) or other assays to confirm the expression/abundance of the glycan of interest. Vector Laboratories offers Glysite™ Scout Glycan Screening Kits (GSK-1000, GSK-2000, GSK-3000). These kits are fully integrated kits for the detection of glycan expression in fixed cells and tissue sections.

Staining Procedure:

1. Prewarm the reagents that need to be at 37°C. Keep the slide incubator moist and preheat it to 37°C. Prewarm TBST and the required number of Coplin jars to 37°C for Step 18 only. If Hematoxylin QS is to be used, remove the bottle from the kit and allow it to warm to room temperature. It can be stored at 4°C but should be at room temperature before applying to samples.
2. For paraffin sections, deparaffinize and hydrate cells and tissue sections through xylenes or other clearing agents and a graded alcohol series.
3. Wash for 5 minutes in tap water.
4. If antigen unmasking is required, perform this procedure using an Antigen Unmasking Solution per manufacturer's standard protocol.
5. Use an ImmEdge® Hydrophobic Barrier PAP Pen to draw around each tissue section.
6. Apply BLOXALL® Blocking Solution to tissue sections and incubate for 10 minutes at room temperature.
7. Wash sections in TBS for 5 minutes.
8. Add Blocking Buffer 1 for 15 minutes at room temperature.
9. Wash sections in TBS for 5 minutes.
10. Prepare Blocking Buffer 2 by diluting Blocking Buffer 2B (25x) in Blocking Buffer 2A. Apply the prepared Blocking Buffer 2 to sections for 30 minutes at room temperature.
11. Wash sections in TBST for 3 minutes, total of 3 washes.
12. Prepare Lectin Solution by diluting Lectin (50x) in Protein Diluent. Apply the prepared Lectin Solution to sections for 30 minutes at room temperature.

13. Wash sections in TBST for 3 minutes, total of 3 washes. If a pause in the protocol is desired, the tissue sections can be stored overnight at 4°C in Protein Diluent. Do not let the sample dry out.

14. Prepare Primary Antibody Solution by diluting either mouse or rabbit primary antibody in Protein Diluent. Apply the prepared Primary Antibody Solution to sections for 30 minutes at room temperature.

15. Wash sections in TBST for 3 minutes, total of 3 washes.

16. Prepare Probe Solution by:
    16.1. Diluting Lectin Probe (50x) in Protein Diluent (Table 1).
    16.2. If using a mouse primary antibody to pair with the lectin, dilute Mouse Probe (50x) in Probe Solution prepared in step 16.1 and proceed to step 17.
    16.3. Alternatively, if using a rabbit primary antibody to pair with the lectin, dilute Rabbit Probe (50x) in Probe Solution prepared in step 16.1 and proceed to step 17.

Table 1. Preparation of Probe Solution for 100 µL.

Lectin Probe (50x)	2 µL
Mouse Probe (50x) or Rabbit Probe (50x)	2 µL
Protein Diluent	96 µL
Total Volume of Probe Solution	100 µL

17. Apply the prepared Probe Solution (which should contain one Antibody Probe, either mouse or rabbit, and one Lectin Probe) for 30 minutes at 37°C. Use a slide incubator for this step and subsequent steps at 37°C.

18. Wash sections in pre-warmed TBST for 5 minutes, total of 3 washes. Keep positive slides and control slides in separate pre-warmed Coplin jars when washing to avoid cross-contamination. Positive samples, single deletions, and double deletions should each have their own Coplin jars. Keep the slides in the Coplin jars at 37°C during the consecutive washes.

19. Prepare Reaction 1 Solution by diluting Buffer 1 (5x) and Enzyme 1 (40x) in distilled water (Table 2). Wait to add enzymes until immediately prior to adding them to the sample. Apply the prepared Reaction 1 Solution for 30 minutes at 37°C.

Table 2. Preparation of Reaction 1 Solution for 100 µL.

Buffer 1 (5x)	20 µL
Enzyme 1 (40x)	2.5 µL
Distilled Water	77.5 µL
Total Volume of Reaction 1 Solution	100 µL

20. Wash sections in TBST for 3 minutes, total of 3 washes.

21. Prepare Reaction 2 Solution by diluting Buffer 2 (5x) and Enzyme 2 (40x) in distilled water (Table 3). Wait to add enzymes until immediately prior to adding them to the sample. Apply the prepared Reaction 2 Solution for 60 minutes at 37°C.

Table 3. Preparation of Reaction 2 Solution for 100 µL.

Buffer 2 (5x)	20 µL
Enzyme 2 (40x)	2.5 µL
Distilled water	77.5 µL
Total volume of Reaction 2 Solution	100 µL

22. Wash sections in TBS for 5 minutes, total of 2 washes.

23. Prepare Detection Solution by diluting ImmPRESS® HRP Reagent (400x) in ImmPRESS® HRP Diluent. Apply the prepared Detection Solution for 30 minutes at room temperature.

24. Wash sections in TBS for 5 minutes, total of 2 washes at room temperature.

25. Prepare ImmPACT DAB Substrate Working Solution by diluting ImmPACT DAB Reagent (33x) in ImmPACT DAB Diluent. Apply the Substrate Working Solution for 8 minutes at room temperature.

26. Wash sections in TBS for 5 minutes.

27. Optionally, rinse slides in tap water and apply Hematoxylin QS to completely cover the tissue section. Incubate sections for 1-10 seconds at room temperature. Rinse slides in tap water. Hematoxylin incubation should be optimized for optimal intensity, as excessive staining may obscure positive isPLA signals.

28. Mount sections in aqueous or permanent mounting media as per standard protocols.

29. Visualize the tissue section using a brightfield microscope, using at least a 20x-40x objective. Puncta, which are small bright chromogenic dots, appear at the site of proximity.

30. After imaging, store the slides at room temperature.

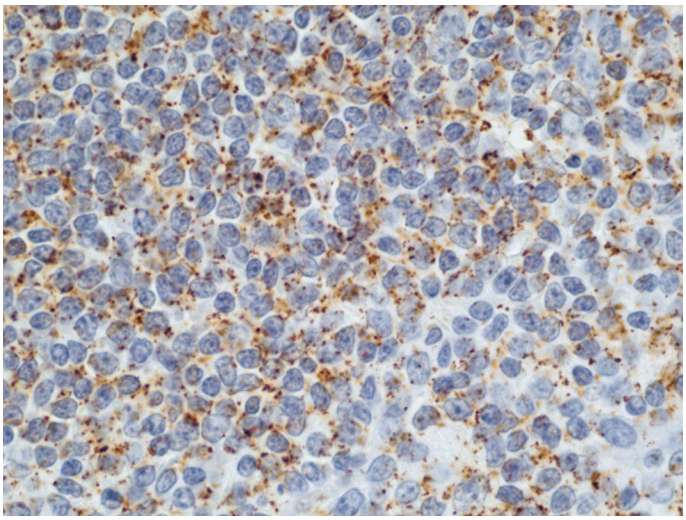
Pro Tips:

- Proper handling procedures should be used to avoid nuclease contamination.
- Use best practices when pipetting to minimize reagents consumption.
- Do not let the sample dry out in between steps.
- Spin-down vials before pipetting.
- The order of addition is critical to obtain appropriate isPLA signals: first apply Lectin Solution, then apply Primary Antibody Solution, lastly add Probe Solution.

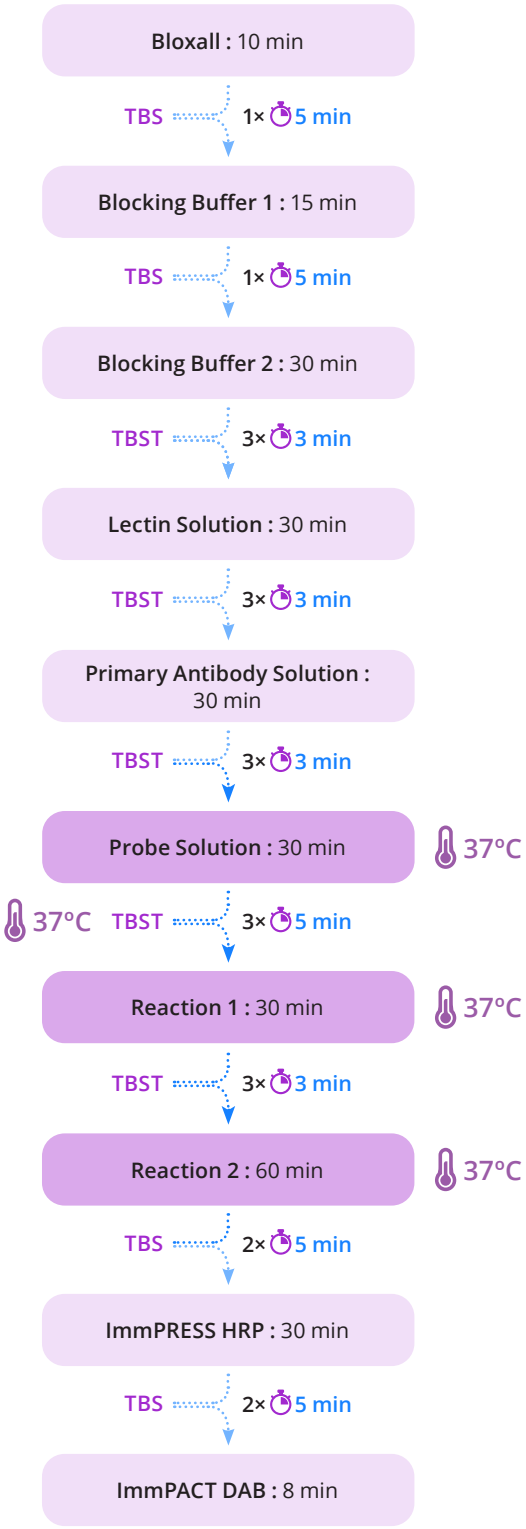
- If a pause in the protocol is desired, the tissue sections can be stored overnight at 4°C in protein diluent after the Lectin Solution in Step 13. Do not let the sample to dry out.
- Completely thaw all buffer mixtures at room temperature and vortex well before use.
- Vortex and spin-down all enzymes (Enzyme 1 and Enzyme 2) before use.
- Keep enzymes on ice or on a frozen cold block until used.
- Wait to add enzymes to Reaction 1 Solution and Reaction 2 Solution until immediately prior to adding them to the sample.
- During wash incubations in Step 18, place Coplin jars with slides in 37°C incubator.
- During wash incubations in Step 18, positive samples, single deletions, and double deletions should each have their own Coplin jars.
- If using fixed cells, ensure cell adhesion to the slide is sufficient to withstand all protocol steps.

Example Data

FFPE Human Tonsil: Glysite™ Explorer *in situ* PLA Glycan Detection Kit with SNA Lectin (50x, GEK-1309) paired with a primary antibody against CD20. Sample was co-stained with Hematoxylin QS.



Workflow



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