

Glysite™ Scout Glycan Screening Kit

Immunofluorescence 594

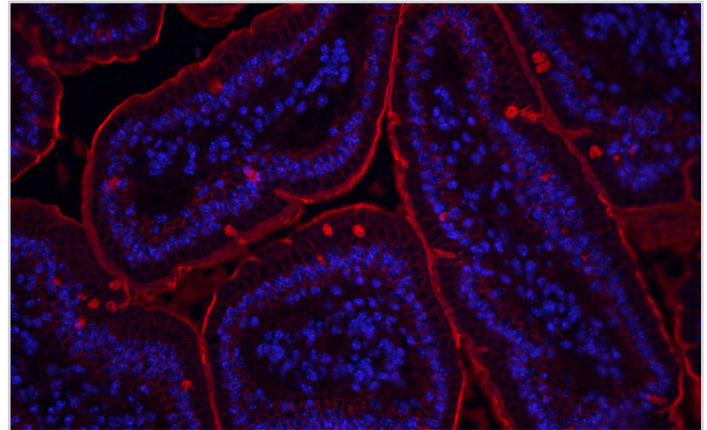
Cat. No.	GSK-2000
Storage	Store reagents in original bottles at 2-8 °C. Do not freeze the kit.
Description	Glysite Scout Glycan Screening Kit, Immunofluorescence 594 is a fully integrated kit for the detection of glycan expression in tissue sections. Included in the kit is a diverse panel of glycan binders (lectins) ideal for the preliminary screening of tissue samples with unknown glycosylation changes with specificity to mannose, complex N-glycan, core O-glycan, fucose, sialic acid, sulfation, GlcNAc, chitin, galactose, and LacNAc. In addition to the glycan binders, the kit includes optimized immunofluorescence reagents for glycan detection, including blocking solutions, DyLight™ streptavidin, and VECTASHIELD Vibrance® Antifade Mounting Medium with DAPI. Validated on human and mouse tissue—including FFPE tissue, across a range of tissue types including colon, lung, spleen, kidney, liver, pancreas, testes, heart, bladder, and uterus—this versatile kit is flexible to meet your needs. Glysite Scout Glycan Screening Kits, Immunofluorescence are available with 3 fluorophore options, 488 (GSK-3000), 594 (GSK-2000), and 649 (GSK-1000).

Kit Components

Product Name	Volume
Carbo-Free Blocking Solution, 10x Concentrate	6 ml
VECTASHIELD Vibrance Antifade Mounting Medium with DAPI	10 ml
Streptavidin Blocking Solution	40 ml
Biotin Blocking Solution	40 ml
Streptavidin, DyLight 594	0.4 ml
AAL (Aleuria Aurantia), Biotinylated	0.025 ml
ECL, ECA (Erythrina Cristagalli), Biotinylated	0.025 ml
GNL (Galanthus Nivalis), Biotinylated	0.025 ml
Jacalin, Biotinylated	0.025 ml
MAL II (Maackia Amurensis II), Biotinylated	0.025 ml
PHA-L (Phaseolus Vulgaris Leucoagglutinin), Biotinylated	0.025 ml
WFA, WFL (Wisteria Fluoribunda), Biotinylated	0.025 ml
WGA (Wheat Germ Agglutinin), Biotinylated	0.025 ml

Preparation of Working Solutions

- Carbo-Free Blocking Solution, 10x Concentrate should be diluted to 1x in DI water. Add 100 µl Carbo-Free Blocking Solution, 10x Concentrate to 900 µl DI water.



FFPE Mouse Intestine: Glysite Scout Glycan Screening Kit, Immunofluorescence 594, biotinylated ECL, ECA (Erythrina Cristagalli), treated with Vector® TrueVIEW® Autofluorescence Quenching Kit. Image was captured with DAPI counterstain.

- All of the biotinylated lectins are provided at 2 mg/ml and should be diluted to 2-10 µg/ml in PBS. Optimal concentration should be determined by the investigator for the target specimen.
- The DyLight streptavidin reagent is provided at 1 mg/ml and should be diluted to 10 µg/ml in PBS.

Staining Procedure

- For paraffin sections, deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series. For frozen sections or cell preparations, fix with acetone or an appropriate fixative for the antigen under study, if necessary.
- Wash for 5 minutes in tap water.
- If antigen unmasking is required, perform this procedure using an Antigen Unmasking Solution, Citrate-based, pH 6.0 (H-3300) or Tris-based, pH 9.0 (H-3301).
- If specimen contains endogenous biotin, biotin receptors, or streptavidin binding sites, perform a streptavidin/biotin block. Incubate sections for 15 minutes with the Streptavidin Blocking Solution. Wash in buffer for 5 minutes. Incubate sections for 15 minutes with the Biotin Blocking Solution. Wash in buffer for 5 minutes.
- Block non-specific binding by incubating sections with 1x Carbo-Free Blocking Solution for 30 minutes.
- Tip off excess blocking solution.

Continued on page 2.

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7. Apply diluted biotinylated lectin to the sections and incubate for 30 minutes.
8. Wash in buffer for 5 minutes.
9. Incubate sections with the DyLight streptavidin reagent for 30 minutes. Keep sections in the dark if possible.
10. Wash in buffer for 5 minutes.
11. Tip off and dab away residual buffer. Apply VECTASHIELD Vibrance Antifade Mounting Medium with DAPI and coverslip sections. Small drop volumes of approximately 25 μ l (per 22 mm x 22 mm coverslip) are recommended.
12. Place mounted samples on a flat, dry surface in the dark and allow the medium to cure. Slides can be viewed 30 minutes after mounting, however optimal antifade performance is achieved after 2 hours.

NOTES

- It is important to run a negative control without detection reagents to examine the autofluorescence of a specimen. If tissue autofluorescence is an issue, use Vector TrueVIEW Autofluorescence Quenching Kit (SP-8400) to diminish the unwanted autofluorescence from non-lipofuscin sources.
- Do not use alternative blocking solutions as they may contain glycoproteins that can interfere with appropriate lectin binding.
- Avoid storing the DyLight streptavidin reagent in strong, direct light.