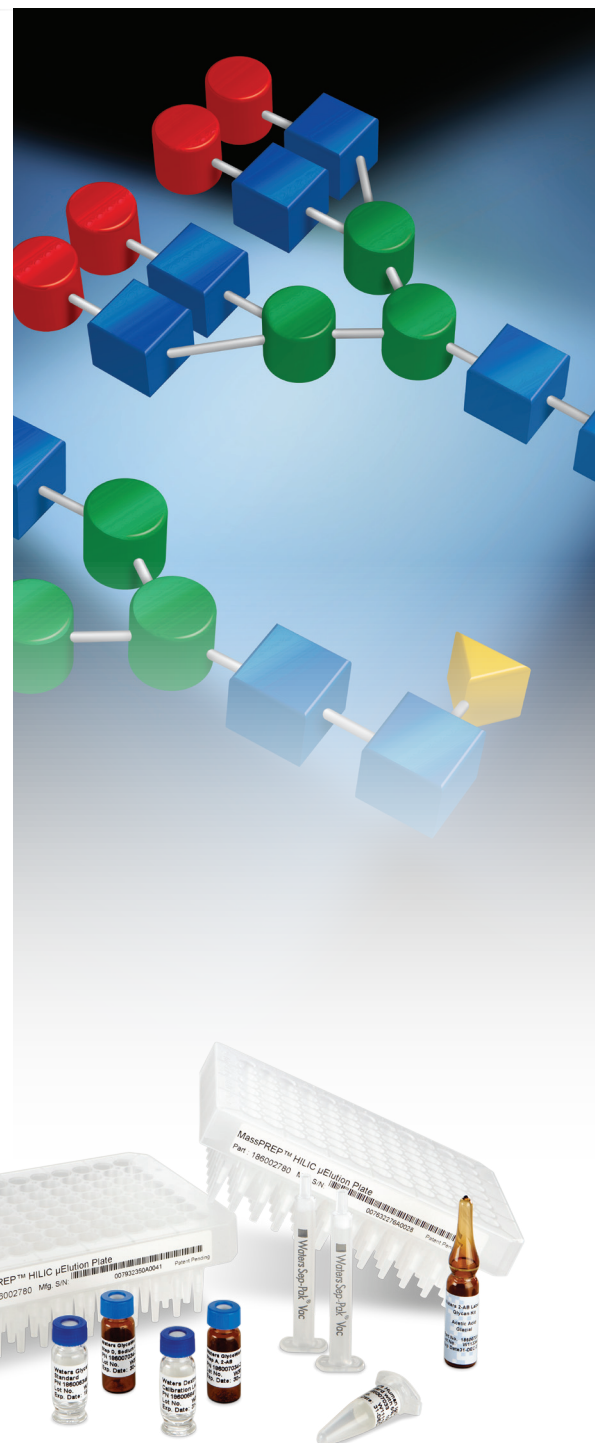
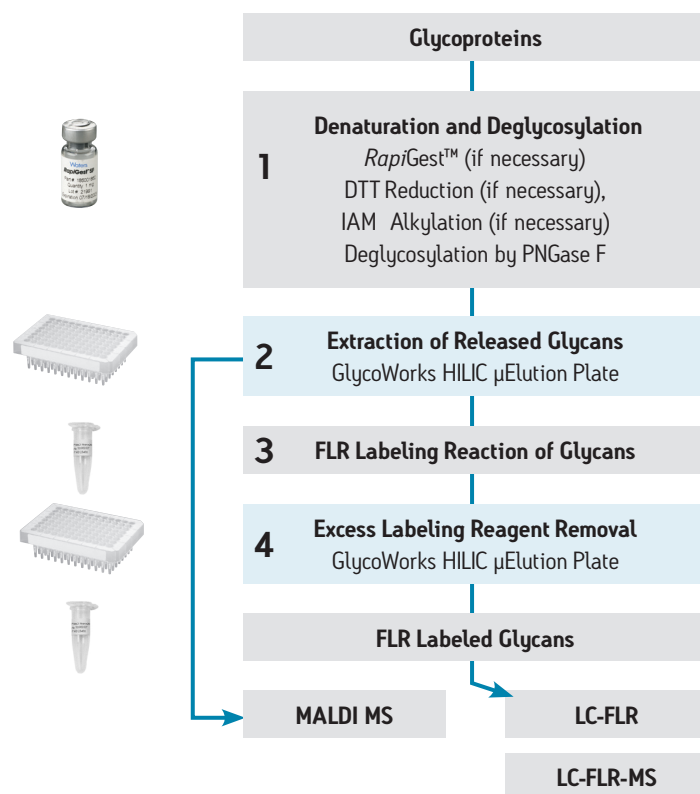


GlycoWorks Sample Preparation Protocol For MALDI

The GlycoWorks™ HILIC μ Elution Plate is ideal for removing contaminants like salts and detergents from hydrophilic analytes, such as carbohydrates, prior to mass spectrometry analysis. It enables elution volumes as low as 25 μ L, thereby eliminating time-consuming evaporation steps. The eluted carbohydrates can be analyzed directly by MALDI MS without any prior derivatization.

General Guideline of Sample Preparation from Glycoprotein to Enrich FLR Labeled Glycans Using Reductive Amination Reaction.



Using the GlycoWorks HILIC μ Elution Plate

Below are general guidelines for utilizing GlycoWorks HILIC μ Elution Plate for extracting, purifying and concentrating carbohydrates. A 96-well collection plate and a waste tray are included as part of the protocol for sample collection and waste disposal.

1. Place GlycoWorks HILIC μ Elution Plate on the vacuum manifold.
2. Condition the sorbent with 200 μ L of Milli-Q[®] water.
Note: Vacuum should be on for this step.
3. Equilibrate device with two x 200 μ L of 90% acetonitrile in Milli-Q water.
Note: Vacuum should be on for this step.
4. Prepare the sample by constituting the sample with 90% acetonitrile in Milli-Q water.
Note: Loss of sample may be observed if the sample is constituted in low concentrations of acetonitrile. 90% acetonitrile is the optimal concentration that should be used.
5. Slowly load sample constituted in 90% acetonitrile in Milli-Q water. Loaded sample volume can range from 100-750 μ L. This step takes 5 to 10 min with no vacuum.
6. Wash sample loaded wells twice with 200 μ L 90% acetonitrile in Milli-Q water.
Note: Vacuum should be on for this step.
7. Elute glycans with two, 50 μ L aliquots 1 mM aqueous Tris-citrate. Tris-citrate is also named Trisodium citrate or sodium citrate tribasic. The 1 mM solution has a pH of approximately 7. For highly sialyated glycans, 10mM Tris-citrate concentration will give better recovery. Also note that the elution buffer may contain up to 25% acetonitrile. To produce a more concentrated solution the glycans may be eluted with two 25 μ L aliquots.
Note: Vacuum should be on for this step.
8. The eluate solutions can be analyzed directly by MALDI.

Sample preparation for MALDI TOF MS analysis:

1. It is recommended to use DHB (2, 5 dihydroxybenzoic acid) as the preferred matrix for glycan analysis. Add 500 μ L of pure ethanol to a 10 mg vial and solubilize the DHB by vortexing. The final concentration is 20 mg/mL.

2. Mix 1 μ L of the glycan solution (desalted as described above) with 1 μ L of the matrix solution. Load 1 μ L onto the MALDI target. Let the droplet dry at ambient temperature until it is completely crystallized. Add 0.8 μ L of pure ethanol to the crystals to form a more homogeneous crystal layer and let dry.
3. Analyze the sample using MALDI MS.

Storage

Plates stored in their original sealed pouch remain stable for long periods. To store unused plates in opened pouches, squeeze the air out of the pouch, fold over the open end of the pouch twice, seal with tape and store in a desiccator.

Note: Dispose of used plates safely in accordance with applicable government or local regulations.

ORDERING INFORMATION

Glycan Sample Preparation Kit and Standards

| Description | Part No. |
|---|-----------|
| GlycoWorks High-throughput Prep Kit* | 176003090 |
| GlycoWorks HILIC μ Elution 96-well Plate | 186002780 |
| RapiGest SF 1 mg vial | 186001860 |
| GlycoWorks Control Standard, 100 μ g vial | 186007033 |
| GlycoWorks Reagent Kit | 186007034 |
| GlycoWorks Single Use Prep Kit* | 176003119 |
| GlycoWorks HILIC 1 cc Cartridge (10/pk) | 186007080 |
| RapiGest SF 1 mg vial | |
| GlycoWorks Control Standard, 100 μ g vial | |
| GlycoWorks Reagent Kit | |
| Glycan Performance Test Standard | 186006349 |
| The Glycan Performance Test Standard is a 2-AB labeled human IgG-like standard that is QC verified to contain the components needed to benchmark and evaluate ACQUITY UPLC BEH Glycan, 1.7 μ m Columns. | |
| Dextran Calibration Ladder | 186006841 |
| The 2-AB labeled, Dextran Calibration Ladder is used to calibrate the HILIC column from retention time to GU values. This calibration ladder provides good peak shape and reliable identification from 2 to 30 Glucose Units. | |

* Available 2Q 2013

Glycan Analysis System Standards

| Description | Usage | Volume | Part No. |
|---|-------------------|--------|-----------|
| Alliance with Fluorescence Qualification Standards Test Kit | C P | | 700002753 |
| (2) 0.5 pg/μL, 1.0 pg/μL, 5.0 pg/μL anthracene in 80/20 acetonitrile/water | | 10 mL | |
| One each: 10.0 pg/μL, 2.5 ng/mL and 2.5 μg/mL, anthracene in 80/20 acetonitrile/water | | 10 mL | |
| Fluorescence Detector Standard Solution | P | | 700003694 |
| 5.0 pg/μL anthracene in 20/80 water/acetonitrile | | 1 mL | |
| Fluorescence Detector Performance Standard Solution | P | | WAT047685 |
| 0.10 mg/L anthracene in 70/30 acetonitrile/water | | 10 mL | |

C Calibration **P** Performance Check

Additional Consumables

| Description | | Part No. |
|----------------------------------|---------------------|------------------------|
| Dextran Calibration Ladder | 200 μg/vial | 186006841 |
| Glycan Performance Test Standard | 228 pmol total/vial | 186006349 |
| RapiGest SF | 1 mg vial/5 pack | 186001861 |
| 96-well collection plate | 96 wells 50/pkg | WAT058943 186002481 |
| μElution Plate Manifold | – | 186001831 |
| SPE Vacuum Pump | 115, 60 Hz | 176002986 |
| | 240, 50 Hz | 176002986 |
| Positive Pressure Manifold | – | 186006961 |

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