Application Manual

FastGlycoProtein™ Isolation Kit WGA Resin

Isolate glycoproteins from complex protein mixtures, and other biological samples in less than 60 minutes.

Cat. No. and Size:
116550900, 10 preps

Storage:
Refrigerated (4° C)

Protocol Revision #116550900-13NOV

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1. Introduction to the FastGlycoProtein™ Isolation Kit, WGA Resin and the FastPrep® Systems

The MP Biomedicals FastGlycoProtein Isolation Kit, isolates glycoproteins from complex protein mixtures including serum and cultured cells as well as animal and plant tissue, microbe and insect lysates using the lectin wheat germ agglutinin (WGA) immobilized on agarose. Lectins are proteins that have a selective affinity for carbohydrate moieties. The WGA lectin preferentially binds N-acetyl glucosamine (GlcNAC) and terminal GlcNAC structures that are commonly present in many serum and membrane glycoproteins. WGA also has affinity for sialic acid.

Protein glycosylation is a common post-translational modification. Asparagine (N-linked) and serine/threonine residues (O-linked) are glycosylated during passage through the endoplasmic reticulum and golgi apparatus in eukaryotic and prokaryotic (i.e., Archaea and Eubacteria) systems. Glycoconjugates are important for immune regulation, inflammation, cell-to-cell adhesion and contact inhibition, cell signaling, protection against proteolytic degradation, and other biological processes.

This kit is easy to use and contains all the necessary components for isolating sialic acid/GlcNAC-containing glycans.. A sample containing up to 1.5 mg of total protein is first placed in lysing matrix A tube, with the Binding/Wash Buffer applied to it. After the tissue/cell lysis is complete, the sample is transferred and applied to the ConA resin bed. Following incubation, the resin is washed and the bound glycoproteins are eluted. Glycoproteins have been successfully isolated from serum as well as HeLa and CHO cell lysates in approximately 40 minutes.

The FastPrep Sample Preparation Instruments including the FastPrep-24™ and FastPrep-96™ are high-throughput homogenizers developed to disrupt thoroughly any tissue or cells through the simultaneous bead-beating and impaction of specialized Lysing Matrix beads on the sample material. The FastPrep Instruments use high-powered complex motions to provide extremely quick and highly reproducible homogenization that surpasses early generation homogenizers, as well as traditional extraction methods using enzymatic digestion, sonication, blending, douncing or vortexing.

Samples are placed into a variety Lysing Matrix Vessel formats containing custom lysing matrix particles designed for optimized disruption of specific sample types. The lysing matrix particles are designed to efficiently lyse organisms such as plant and animal tissue, cultured cells, bacteria, yeast and fungi, including historically difficult sources such as eubacterial spores and endospores, algae, and nematodes, while in the presence of a

specially formulated lysis solution. The FastPrep Instruments lyse samples quickly and efficiently, most in 40 seconds or less, all while preserving the protein's functionality and biological activity.

2. Kit Components, Storage and User Supplied Materials

2.1 FastGlycoProtein™ Isolation Kit Components

Lysing Matrix A	10 x2 mL Tubes
WGA Lectin Resin	1.1 mL settled, as slurry
Binding/Wash Buffer. 5X solution	6.5 mL
Elution Solution	5 mL
Spin Filter with caps	10 each
Catch Tubes	20 each
User manual	1 each
MSDS (Online: www.mpbio.com)	1 each
Certificate of Analysis	1 each

2.2 Storage

All FastGlycoProtein™ Isolation Kit, ConA Resin should be stored at 4°C immediately upon receipt. The Kit is shipped ambient. The kit reagents are guaranteed for up to one year from the date of purchase of the kit.

2.3 User Supplied Materials

FastPrep Instrument (optional)
Benchtop microcentrifuge capable of 10,000 xg rcf
Inverting lab shaker or rocker
Sterile water

3. Important Considerations Before Use

3.1 Proteases/metal chelators

If needed, add protease inhibitors to samples; however, avoid cocktails containing EDTA or other metal chelators.

3.2 End-product compatibility

Samples purified with this kit are compatible with 1-D gel electrophoresis, and Total Protein (Bradford type) Assays. Other downstream applications may require sample processing to remove incompatible substances in the Elution Buffer. To quantify protein using a BCA type Assay, desalt sample using a 5 ml desalting column. . For 2-D gel electrophoresis, clean up sample using the precipitation or dialysis or other purification technique.

3.3 Abundant Protein Removal

Removing albumin and IgG from serum samples improves isolation of less-abundant glycoproteins for mass spec applications.

4. Safety Precautions

Some of the supplied kit reagents contain components that, when in contact with human tissue, may cause irritation. Wear personal protective equipment to prevent contact with the skin or mucus membranes (gloves, lab coat, and eye protection) at all times when using this product. Consult the Material Safety Data Sheet at www.mpbio.com for additional details. This product is for research purposes only.

5. Protocol

IMPORTANT NOTE BEFORE STARTING PROTOCOL:

Equilibrate the Binding/Wash and Elution Buffers to room temperature.

Sample Preparation

1. For serum samples or biological lysates from sonication, douncing, or other chemical digest, dilute sample containing 1 to 1.5 mg of total protein 4:1 with 5X Binding/Wash Buffer stock solution (e.g., mix approximate 400 µL of volume of sample with 100 µL 5X Binding/Wash Buffer). The total volume, including dilution, must not exceed 800 µL.

Optional: For mechanical cell lysis using FastPrep instruments, place the sample containing up to 1.5mg of proteins in a Lysing Matrix A tube. Dilute sample 4:1 with 5X Binding Wash Buffer stock solution (e.g., mix approximate 400 µL of volume of sample with 100 µL 5X Binding/Wash Buffer). The total volume, including dilution, must not exceed 800 µL. Process sample in a FastPrep in accordance to the recommended settings for the sample type (see Appendix 1),

Isolation of Glycoproteins

1. Prepare the 1X Binding/Wash Buffer dilute 460 µL 5X Binding/Wash Buffer with 1,840 µL of ultrapure water. This is sufficient volume to process one sample.

2. Insert a column into a collection tube.

3. Gently swirl the bottle of WGA Lectin Resin to obtain a homogeneous suspension. Use a wide-bore or cut pipette tip to transfer 200 µL of 50% resin slurry to the column

4. Centrifuge 1 minute at 1,000 ×g and discard the storage buffer. Reuse the catch tube through step 12.

5. Replace the column in the same catch tube and add 200 µL 1X Binding/Wash Buffer to the resin Close the top cap and centrifuge column for 1 minute at 1,000 ×g and discard rinse. Repeat this step two times. (3x washes are necessary to condition the resin.)

6. Place bottom cap on column and add sample, from Sample Preparation step (total sample volume not to exceed 800 µL) to the resin. Close the top cap.

7. Incubate column for 10 minutes at room temperature with end-over-end mixing using a rotator (e.g., Labquake® Shaker by Thermolyne). Alternatively, rock back and forth on a rocking platform.

8. Remove top cap and then bottom cap from column. Place column in the catch tube, and replace top.

Note: Remove top cap first and then quickly remove bottom cap and place immediately in catch tube in order to prevent sample from leaking from the bottom of the column.

9. Centrifuge column for 1 minute at 1,000 ×g and discard flow-through.

Note: If desired, save flow-through for SDS-PAGE or protein assay analysis as this will contain all other non-glycosylated proteins if present.

10. Reinsert column and add 400 µL 1X Binding/Wash Buffer to the resin. Cap column and centrifuge for 1 minute at 1,000 × g and discard flow-through. Repeat this step.

11. Place bottom cap on column and add 400 µl 1X Binding/Wash Buffer to the resin. Cap column and incubate for 5 minutes at room temperature with end-over-end mixing using a rotator.

12. Remove top cap and then bottom cap from column. Place column in the collection tube, and replace top cap. Centrifuge column for 1 minute at 1,000 × g and discard rinse.

13. Repeat Steps 11 and 12.

14. Replace bottom cap on column. Add 200 µl Elution Buffer to resin and cap column. Incubate column for 5 minutes at room temperature with end-over-end mixing using a rotator.

15. Remove top cap and then bottom cap from column. Place column in a new catch. Replace top cap and centrifuge column for 1 minute at 1,000 × g.

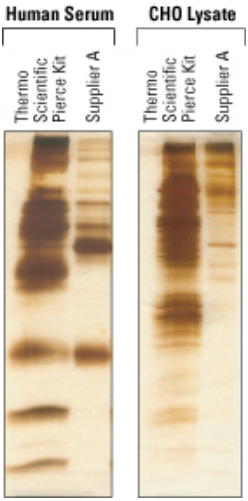
16. Carefully set aside the catch tube and remove top cap.

17. Repeat Steps 14-16. Pool eluate #2 in the same catch tube that contains the first elute. Store eluted glycoproteins on ice for immediate use or freeze for later analysis.

6. Troubleshooting

Problem	Possible Cause	Solution
Low glycoprotein recovery in elution fraction	Some glycoproteins have high affinity for the immobilized WGA and will not elute with Elution Buffer	Increase incubation with elution buffer to 10 minutes or boil resin in 200 µl of SDS-PAGE sample buffer for 5 minutes and then centrifuge column in a 2 ml tube for 1 minute at 1,000 × *g* to collect eluate **Note:** Boiling the resin results in detachment of some lectin and also may release non-specifically bound proteins
Glycoprotein is not binding to the resin	Sample contains metal chelator(s)	Confirm EDTA or other metal chelators are not present in the sample

7. Example Data: Glycoprotein Isolation from Human Serum and CHO Lysate Samples and Gel Electrophoresis



Glycoprotein isolation from human serum and cell lysate – performance comparison of kits using WGA resin. CHO lysate and human serum samples were processed with the FastGlycoProtein Isolation Kit, WGA Resin™ and with other commercially available WGA resins. An equivalent amount of total protein was applied to each resin. Eluted glycoprotein fractions were normalized by volume and resolved on 8-16% polyacrylamide gels. (a) Eluted glycoprotein fraction from applied CHO lysate (b) Eluted glycoprotein fraction from applied human serum

8. Recommended Reference Format for Publications

Glycoproteins were isolated from (specific sample) using the FastGlycoProtein™ Isolation Kit, WGA Resin and the FastPrep(insert model)™ Instrument (MP Biomedicals, Santa Ana, CA).

9. References

Cooper, C.A., *et al.* (2001) GlycoSuiteDB: A new curated relational database of glycoprotein glycan structures and their biological sources. Nucl. Acid. Res. 20(1):332-5.

Cummings, R.D. (1997). Affinity chromatography of oligosaccharides and glycopeptides. Affinity Separations: A Practical Approach (Matejschuk, P., Ed.), Oxford Univ. Press, London, pp. 123-139.

Ghosh, D., *et al.* (2004). Lectin affinity as an approach to the proteomic analysis of membrane glycoproteins. J. Proteome Res. 3:841-850.

Young, N.M., *et al.* (2002). Structure of the N-linked glycan present on multiple glycoproteins in the gram-negative bacterium, *Campylobacter jejuni. J. Biol. Chem.* 277:42530-9.

10. Technical Support

For technical support with this product please contact our MP Biomedical's Technical Support Team at 1-800-854-0530, by email at biotech@mpbio.com, or visit us online at www.mpbio.com for live support.

11. Related Products

Description	Size	Catalog #
Coomassie*Brilliant Blue R250		1104821636
Ammonium Sulfate, Ultra-Pure™		1104808211
Biotrans™ PVDF Transfer Membranes		
FastPrep-96™ Instrument		116010500
FastPrep 24™ Instrument		116004500
FastDNA Kit™	100 preps	116540400
FastDNA™ SPIN Kit	100 preps	116540600
FastDNA™ SPIN Kit for Soil	50 preps	116560200
FastDNA™ 50ml SPIN Kit for Soil	10 preps	116570200
FastRNA™ Pro Soil-Direct Kit	50 preps	116070050
FastRNA™ Pro Soil-Indirect Kit	50 preps	116075050
FastRNA™ Pro Red Kit (Yeast & Fungus)	50 preps	116035050
FastRNA™ Pro Green Kit (Plant & Animal)	50 preps	116045050
FastRNA™ Pro Blue Kit (Bacteria)	50 preps	116025050
FastProtein™ Blue Matrix	50 preps	116550400
FastProtein™ Red Matrix	50 preps	116550600

12. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's
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Ready-to-use Protocols For DNA, RNA And Protein Isolation From Any Sample

- Rapid and reproducible sample lysis and purification process
- No cross contamination with the closed lysing matrix tubes
- Increased yields of high quality DNA, RNA and proteins
- Integrity and size of DNA, RNA and proteins are retained
- Nucleic acids and proteins are ready-to-use in downstream application

FastDNA Kits

FastDNA® Kit and FastDNA® Spin Kit
116540400 - 116540600 respectively (100 preps)
- Lyse and isolate DNA in less than 30 minutes
- Plant, animal, yeast, fungal and microbial samples
- No hazardous organic reagents required
- SPIN filters streamline silica handling (FastDNA Spin Kit)

FastDNA® Spin Kit for Feces
116570200 (50 preps)
- Lyse and isolate PCR-ready DNA in less than 30 minutes
- Variety of stool sample types
- No hazardous organic reagents required
- SPIN filters streamline silica handling

FastDNA® 50ml Spin Kit for Soil
116560600 (10 preps)
- Process low-microbial containing soil samples.
- Lyse and isolate PCR-ready DNA in less than 60 minutes
- Variety of soil and environmental sample types
- No hazardous organic reagents required
- SPIN filters streamline silica handling

FastRNA® Pro Blue Kit
116025050 (50 preps)
- For use with gram positive and gram negative bacteria
- Lyse up to 1010 cells per 2mL tube
- Lysis and isolation with single-phase organic solution in less than 90 minutes

FastRNA® Pro Red Kit
116035050 (50 preps)
- For use with yeast cells and fungal tissue
- Lyse up to 1010 cells per 2mL tube
- Lysis and isolation with single-phase organic solution in less than 90 minutes

FastRNA® Pro Green Kit
116045050 (50 preps)
- For use with all plant and animal samples
- Lyse 50-100 mg tissue per 2mL tube
- Lysis and isolation with single-phase organic solution in less than 90 minutes

FastRNA® Pro Soil-Direct Kit and
FastRNA® Pro Soil-Indirect Kit
116070050 - 116075050 respectively (50 preps)
- Isolate RNA from soil samples (direct kit) and washed soil (indirect kit) in less than 2 hours
- Variety of soil and environmental sample types
- RNA protected during and after processing
- Humic acids reduced to allow uninhibited RT-PCR
- Includes additional reagents for even further purification if necessary
- SPIN filters streamline silica handling

FastProtein™ Blue Matrix
116550400 (50 preps) - 116550500 (100 preps)
- Release of proteins from gram positive and gram negative bacteria in 40 seconds
- Protein extracts are ready for immediate electrophoresis or purification
- Ideal for optimizing induction conditions

FastProtein™ Red Matrix
116550600 (50 preps) - 116550700 (100 preps)
- Release of proteins from yeast cells and fungi in 40 seconds
- Protein extracts are ready for immediate electrophoresis or purification
- Ideal for optimizing induction conditions

Appendix 1

General recommendations for processing samples with FastPrep Instruments

The fill volume in the lysing matrix tube after the addition of the Cell Lysis Solution to the sample should allow sufficient air space in the sample tube for efficient FastPrep® Instrument processing. MP Biomedicals recommends using 100 – 200 mg of starting material as long as there is between 250 – 500 µL of empty space in the tube. Sample loss or tube failure may result from overfilling the matrix tube. The matrix tube caps must be secure, but not over-tightened, to prevent sample leakage. If the sample is too large for processing in a single tube, divide the sample and process using multiple tubes.

MP Biomedicals' Lysing Matrix particles and tubes have been rigorously tested and validated in the FastPrep® Instrument. The use of other products with the FastPrep® Instrument is not recommended and may result in sample loss or instrument failure. A single 40 second run at a speed setting of 6.0 in the FastPrep® Instrument is sufficient to lyse

almost all samples. If the user experimentally determines that additional processing time is required, the sample should be incubated on ice in the Lysing Matrix A tube for at least 2 minutes between successive FastPrep® Instrument homogenizations to prevent overheating the sample and tube.

MP Biomedicals recommends that all researchers begin the protocol with the Lysing Matrix A as supplied in the kit (garnet matrix and single sphere). If lysis is inefficient even after multiple runs of 40 seconds, an additional ¼ inch ceramic sphere (provided) can be added on top of the sample. Depending on the sample, lysis and/or yield may or may not improve and shearing of existing genomic DNA may begin to occur. Samples with 2 spheres should be processed carefully in order to balance increased yield and lysis against increased DNA shearing by varying speed and/or time settings.

General Speed, Time, and Lysing Matrix Recommendations per Sample Type

Sample Name	Sample Type	Quantity	Lysing Matrix	FastPrep Speed	FastPrep Time
Alpowa Wheat	Leaf Tissue	75 mg	D	6.0	40 sec
Arabidopsis thaliana	Fresh Leaves	200 mg	D	6.0	2 x 40 sec
Bartlett Pear	Leaf Tissue	50 mg	D	6.0	40 sec
Classic Oat	Seed	100 mg	A	6.0	40 sec
Corn	Leaf Tissue	100 mg	D	6.0	40 sec
Crest Barley	Root	300 mg	A	6.0	40 sec
Kaybonnet Rice	Seed	100 mg	A	6.0	40 sec
Klages Barley	Root	300 mg	A	6.0	40 sec
Tobacco	Leaf Tissue	75 mg	D	6.0	40 sec
Lafitte Rice	Leaf Tissue	75 mg	D	6.0	40 sec
Lafitte Rice	Sprout Leaf	100 mg	D	6.0	2 x 30 sec
Soybean	Seed	100 mg	A	6.0	40 sec
Corn	Seed	100 mg	A	6.0	40 sec
Oat FL 502	Leaf Tissue	75 mg	D	6.0	40 sec
Tam Wheat	Leaf Tissue	75 mg	D	6.0	40 sec
Tam Wheat	Root	80 mg	A	6.0	40 sec
Tomato, Early Girl	Leaf Tissue	75 mg	D	6.0	4 x 30 sec
Pine	Needle	100 mg	A	6.0	30 sec
Crest Barley	Root	300 mg	A	6.0	40 sec
Human	Lung	50 mg	D	6.0	4 x 30 sec.
Human	Breast	80 mg	D	6.0	2 x 30 sec
Human	Kidney	50 mg	D	6.0	40 sec
Human Thyroid Tumors	Thyroid Tumors	100 mg	A	6.0	3 x 30 sec
Mouse	Eye	10 mg	D	6.0	4 x 30 sec.
Mouse	Heart	70 mg	D	6.0	4 x 30 sec.
Mouse	Femur	40 mg	A	6.0	4 x 30 sec.
Mouse	Leg Muscle	50 mg	D	6.0	40 sec
Mouse	Intestine	50 mg	D	6.0	40 sec
Mouse	Tail	100 mg	A	6.0	4 x 30 sec.
Mouse	Spleen	70 mg	D	6.0	40 sec
Mouse	Liver	50 mg	D	6.0	40 sec
Mouse	Brain	50 mg	D	6.0	40 sec
Mouse	Pancreatic cells (bHC9)	10^7 cells	D	6.0	40 sec
Ground Water	Wastewater	1.0 mL	E	6.0	40 sec
Sediment	Marine Sediment	500 mg	E	5.5	2 x 40 sec
Sediment	Soil/Rock	50 mg	E	5.5	2 x 30 sec
Soil	Sandy Sample	50 mg	E	4.0	4 x 30 sec
Soil	Litter	50 mg	E	5.5	40 sec
Soil	Soil from Grassland	500 mg	E	5.5	2 x 30 sec
Soil	Rhizosphere	500 mg	E	6.0	40 sec
Sediment	Marine Sediment	500 mg	E	5.5	2 x 40 sec
Soil	Asphalt-permeated Soil	500 mg	E	6.0	40 sec

Application Manual

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