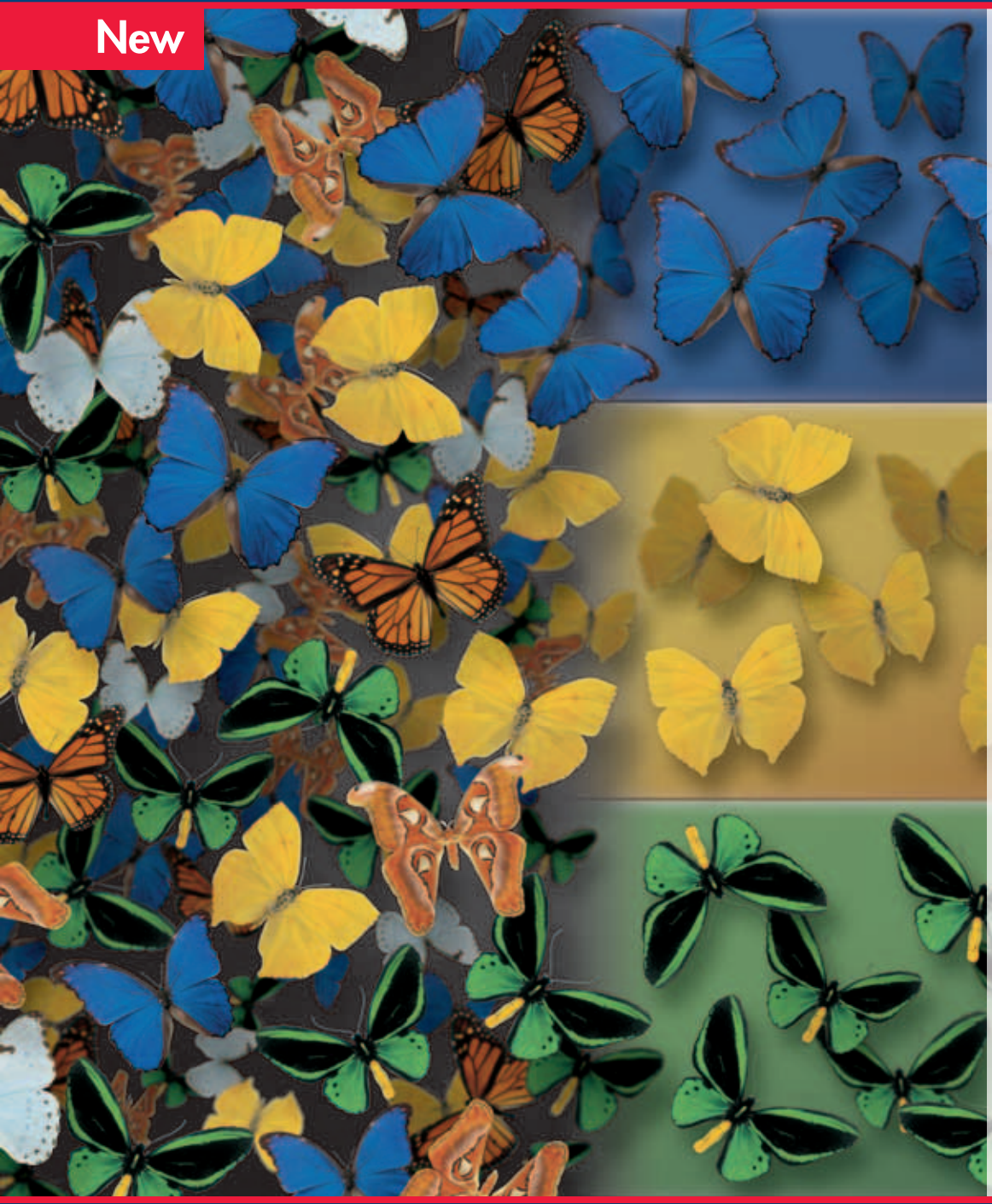
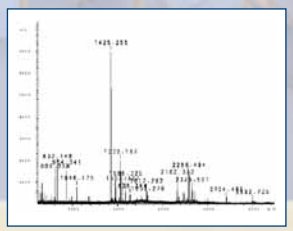
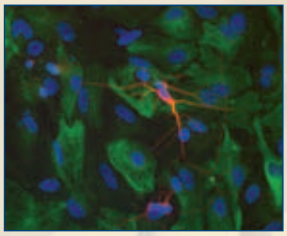
Integrated Solutions — Proteomics

Qproteome™ Kits from QIAGEN — Making the Proteome More Manageable

New







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Qproteome kits — making the proteome more manageable

A major problem facing protein researchers is the sheer complexity of the proteome. Purification and quantification of low-abundance proteins is especially challenging. To address these problems, QIAGEN has used its expertise in sample preparation to develop the Qproteome range of kits for standardized and reproducible protein fractionation and depletion.

Qproteome kits offer:

- Reduced sample complexity according to highly specific separation criteria — efficient isolation of targeted subsets of proteins for easier analysis of low-abundance species
- Simple, easy-to-use kit formats — no specialized equipment required
- Highly reproducible, standardized separation — reliable results, time after time
- Intact, native-conformation proteins — suitable for enzymatic assays and other activity-based downstream applications

Table 1. The Range of Qproteome Kits and their Properties

Qproteome kit	Protein categories	Fractions isolated
Soluble Protein Separation Kit	Whole proteome	6 fractions according to protein solubility
Cell Compartment Kit	Organelle-/cell compartment-specific	Cytosolic, membrane, nuclear, and cytoskeletal proteins
Phosphoprotein Purification Kit	Phosphorylated proteins	Phosphorylated and unphosphorylated proteins
Total Glycoprotein Kit	Glycosylated proteins	Two separate enriched fractions containing glycoproteins carrying the lectin-specific glycan moiety
Mannose Glycoprotein Kit	Glycosylated proteins (mannose rich)	Three separate enriched fractions containing glycoproteins carrying the lectin-specific glycan moiety
Sialic Glycoprotein Kit	Glycosylated proteins (sialic acid rich)	Three separate enriched fractions containing glycoproteins carrying the lectin-specific glycan moiety
O-Glycan Glycoprotein Kit	Glycosylated proteins (T-antigen type glycan-rich)	Two separate enriched fractions containing glycoproteins carrying the lectin-specific glycan moiety
Albumin/IgG Depletion Kit	Human serum and plasma proteins	Albumin and IgG-free serum or plasma sample
Nuclear Subfractionation Kit	Nuclear and nucleic acid binding proteins	Cytosolic proteins; subfractionated nucleic acid binding proteins; "insoluble" nuclear proteins (e.g., histones)

A general-use kit for reproducible fractionation and enrichment

Protein researchers often need to focus on a single protein or protein complex. Based on their solubility, the Qproteome Soluble Protein Separation Kit separates proteins into six distinct fractions (see Figure 1) in a simple and highly reproducible procedure.

The Qproteome Soluble Protein Separation Kit offers:

- Highly reproducible fractionation of any cell lysate
- Simple procedure — just add fractionation buffer and spin
- Fractions containing active proteins, ready to use in downstream applications

Efficient Fractionation of a Cell Lysate

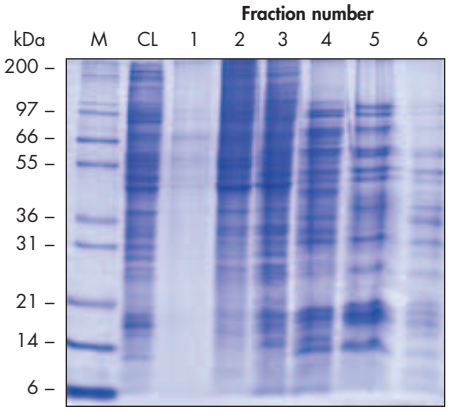


Figure 1 Fractionation of an NIH-3T3 cell lysate sample using the Soluble Protein Separation Kit. **CL**: cleared lysate; **M**: markers.

Separate proteins according to their cellular location

Compartmentalization enables cells to perform sophisticated intracellular chemistry, and the proteins that perform this chemistry are often confined to a distinct structure within the cell.

The Qproteome Cell Compartment Kit enables researchers to:

- Efficiently and reproducibly separate proteins that are found in the cytosol, membranes, nucleus, or cytoskeleton (Figure 2)
- Monitor localization of proteins under different cellular growth conditions
- Enrich a particular subset of proteins from a cell compartment prior to further purification or analysis

Specific Separation of Marker Proteins

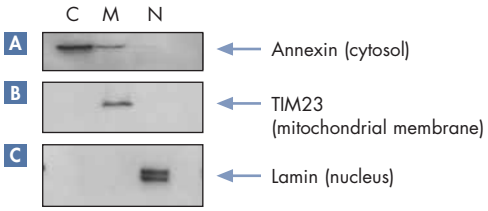
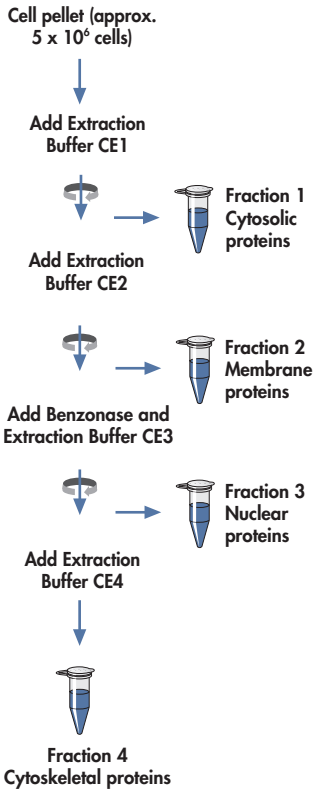
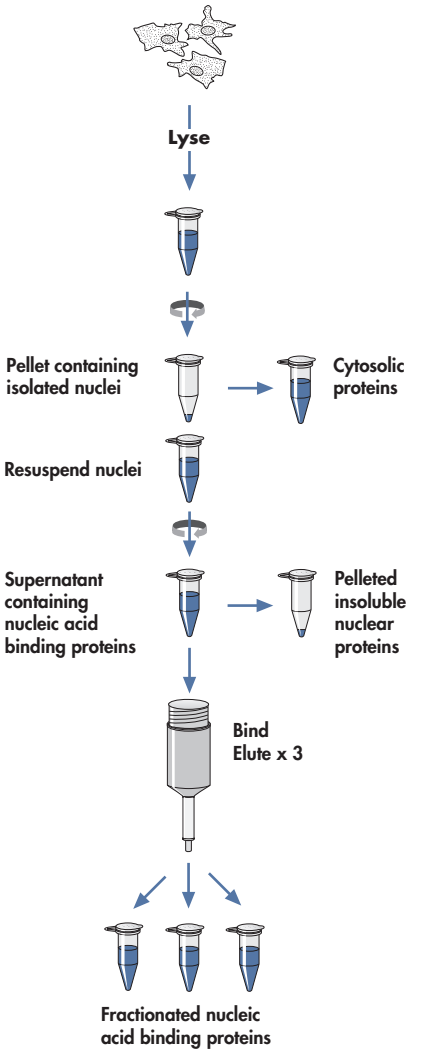


Figure 2 Western blots of fractionated NIH 3T3 cells. Protein (20 µg) from the cytosolic (**C**), membrane (**M**), and nuclear (**N**) fractions was separated by SDS-PAGE. After Western blotting, proteins specific to each fraction were detected using **A** annexin, **B** TIM23, and **C** lamin antibodies, and an HRP-conjugated secondary antibody with chemiluminescent detection.

Cell Compartment Kit Fractionation Procedure



Nuclear Protein Fractionation Procedure



Efficiently isolate and subfractionate active nucleic acid binding proteins

The identification and study of nuclear proteins — especially nucleic acid binding proteins (e.g., transcription factors) — is important for an understanding of genome regulation and function. The large number (>1000) and low abundance of some species makes fractionation a prerequisite for analysis of these important proteins using current methods.

The Qproteome Nuclear Subfractionation Kit offers:

- High-purity nuclear protein fractions free of cytosolic proteins
- Subfractionation of nucleic acid binding proteins (e.g., transcription factors and other proteins involved in gene regulation, Figures 3 and 4)
- A huge reduction in sample complexity, greatly facilitating study of low-abundance nuclear proteins

Reproducible, Efficient Separation of Marker Proteins

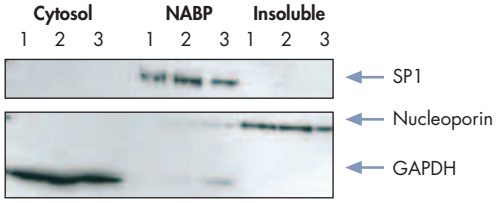


Figure 3 Three cell lysate preparations were processed in parallel using the Qproteome Nuclear Subfractionation Kit. Fractions were separated by SDS-PAGE. Fraction-specific markers (GAPDH, cytosolic fraction; transcription factor SP1, nucleic acid binding protein fraction [NABP]; and nucleoporin, insoluble fraction) were detected using protein-specific antibodies in a Western blotting procedure.

Isolation of an Active Transcription Factor

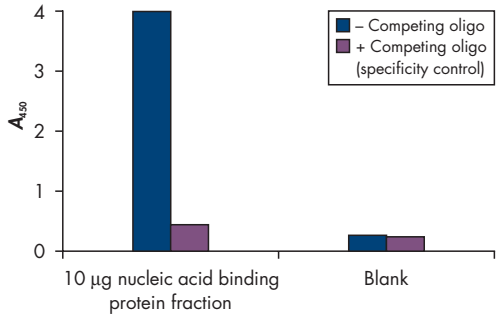


Figure 4 A biotinylated DNA oligo containing a specific transcription-factor binding sequence was immobilized on a streptavidin-coated 96-well plate. NX1 extraction buffer (Blank) or 10 µg nucleic acid binding protein fraction was added, washed, and detected colorimetrically in an ELISA procedure using a transcription-factor specific antibody. Specificity of binding was demonstrated by addition of a 10x excess of non-biotinylated oligo that was able to displace the transcription factor.

Fast, efficient albumin and IgG depletion of serum and plasma samples

Body fluids, such as serum and plasma, are widely used in clinical research and diagnostic procedures. A major problem in analyzing the makeup of these samples is the huge dynamic range of concentrations of their constituent proteins.

The Qproteome Albumin/IgG Depletion Kit provides:

- Efficient removal of human albumin and IgG to facilitate analysis of less abundant proteins (Figure 5)
- Highly specific depletion through use of immobilized monoclonal antibodies
- Easy-to-use spin column format in a fast procedure

Albumin/IgG Depletion Facilitates Analysis of Low-Abundance Proteins

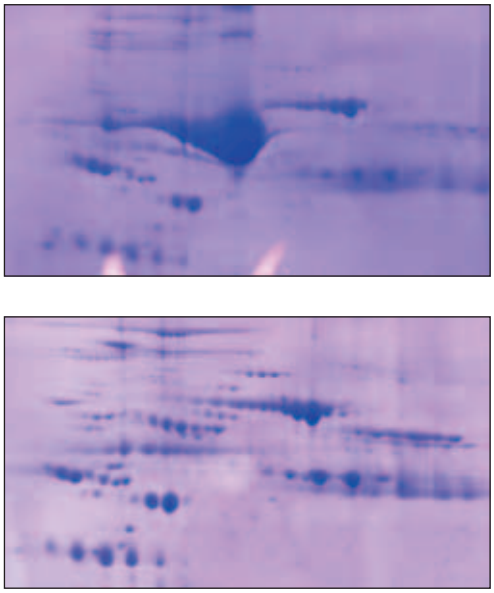


Figure 5 Coomassie® stained 2-D PAGE gels showing non-depleted (upper panel) and depleted (lower panel) plasma samples.

Separation of phosphorylated and unphosphorylated proteins

Phosphorylation of proteins plays a vital role in cell signaling, oncogenesis, apoptosis, and immune disorders. The PhosphoProtein Purification Kit uses an affinity resin to separate unphosphorylated proteins (which are contained in the column flow-through) from phosphorylated proteins (which are eluted using a phosphate buffer). This separation greatly facilitates investigation of the phosphorylation status of entire cells and specific proteins.

The QIAGEN® Phosphoprotein Purification Kit offers:

- Highly specific separation of phosphorylated and unphosphorylated proteins for facilitated analysis of either fraction (Figure 6)
- Cell signaling studies without the need for radioactivity
- A complete system, including columns, buffers, and reagents

Highly Specific Separation of Phosphorylated Proteins

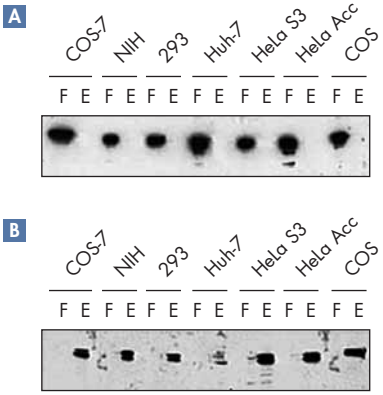


Figure 6 Protein-specific immunodetection of A unphosphorylated HSP-60 protein, and B phosphorylated p44 and p42 mitogen-activated protein kinase (MAPK) proteins. **F**: flow-through; **E**: eluate fractions. The antibody used to detect MAPK recognizes an epitope containing phosphorylated residues at Thr202 and Tyr204 in the p44 (upper band) and p42 (lower band) MAPK amino acid sequences. The absence of unphosphorylated HSP-60 in the eluate fraction and the absence of phosphorylated MAPK in the flow-through fraction demonstrate the complete separation of phosphorylated proteins using the PhosphoProtein Purification Kit.

Highly Specific Glycoprotein Fractionation Using Lectin Spin Columns

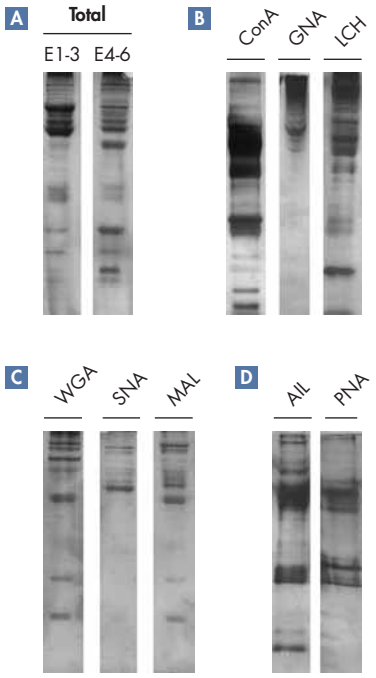


Figure 7 Glycoproteins were fractionated from serum using the different lectin spin columns in glycoprotein fractionation kits and analyzed by SDS-PAGE followed by silver staining. A Elution steps 1–3 and 4–6 from Total Lectin Spin Columns in the Total Glycoprotein Kit. B Eluted glycoproteins from ConA, GNA, and LCH Spin Columns in the Mannose Glycoprotein Kit. C Eluted glycoproteins from WGA, SNA, and MAL Spin Columns in the Sialic Glycoprotein Kit. D Eluted glycoproteins from AIL and PNA Spin Columns in the O-Glycan Glycoprotein Kit.

Highly Specific Isolation of Glycoproteins

One of the most common post-translational protein modifications is glycosylation, which plays a vital role in a wide range of cellular processes such as cell adhesion and signaling, stabilization of protein structure and function, protein trafficking and sorting, and oncogenesis.

Qproteome Glycoprotein Fractionation Kits offer:

- Highly specific separation of glycoproteins according to the structure of their glycan moieties (Figures 7 and 8)
- Profiling of glycoproteins in cells grown under different conditions or in different disease states
- A wide selection of lectin columns for comprehensive and precise glycoprotein characterization

Comprehensive and Precise Analysis of Glycan Moieties

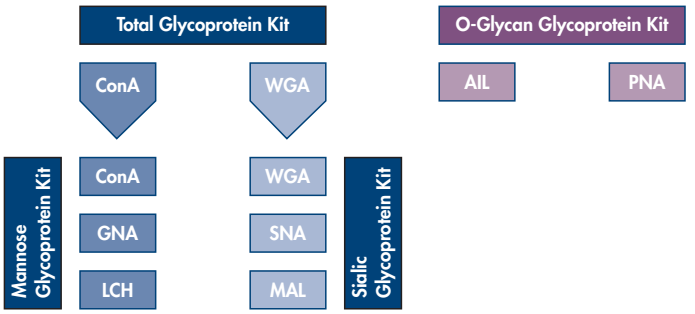
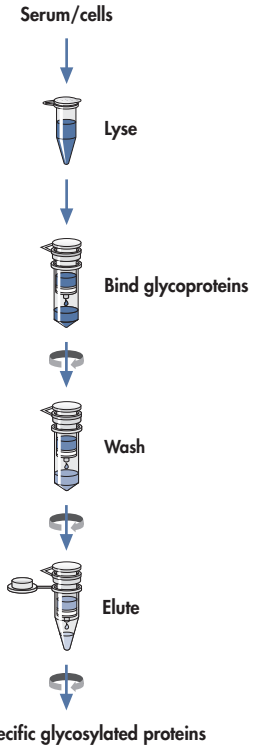


Figure 8 Initial analysis of glycoproteins can be carried out using the Total and O-Glycan Glycoprotein Kits. Depending on which lectin column binds a protein of interest, further studies on its precise nature can be performed using either the Mannose or Sialic Glycoprotein Kit.

Glycoprotein Fractionation Procedure



Ordering Information

Product	Contents	Cat. no.
Qproteome Soluble Protein Separation Kit	For 10 soluble protein fractionations: Fractionation Buffer, Precipitation Reagents, Protease Inhibitor Solution, Benzonase®	37512
Qproteome Cell Compartment Kit	For 10 subcellular fractionations: Extraction buffers, Protease Inhibitor Solution, Benzonase	37502
Qproteome Nuclear Subfractionation Kit	For 6 nuclear protein preparations: Buffers, Reagents, Nuclear protein Fractionation Columns (6), Nuclear Protein Fractionation Resin, Protease Inhibitor Solution, Benzonase	37531
Qproteome Albumin/ IgG Depletion Kit	For albumin/IgG depletion of 6 serum or plasma samples: Albumin/ IgG Depletion Spin Columns (6)	37521
Qproteome Total Glycoprotein Kit	For 6 total glycoprotein preps: Buffers, Lectin Spin Columns (6), Detergent Solution, Protease Inhibitor Solution, Collection Tubes (6 x 2 ml)	37541
Qproteome Mannose Glycoprotein Kit	For 6 mannose glycoprotein preps: ConA, GNA, and LCH Lectin Spin Columns (2 each); Buffers; Detergent Solution; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37551
Qproteome Sialic Glycoprotein Kit	For 6 sialic acid glycoprotein preps: WGA, SNA, and MAL Lectin Spin Columns (2 each); Buffers; Detergent Solution; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37561
Qproteome O-Glycan Glycoprotein Kit	For 6 O-glycan glycoprotein preps: AIL and PNA Lectin Spin Columns (3 each); Buffers; Protease Inhibitor Solution; Collection Tubes (6 x 2 ml)	37571
PhosphoProtein Purification Kit (6)	6 PhosphoProtein Purification Columns, 6 Nanosep® Ultrafiltration Columns, Reagents, Buffers	37101

Find out more about how Qproteome kits can facilitate your proteomic research at www.qiagen.com

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