Whatman®

interchim

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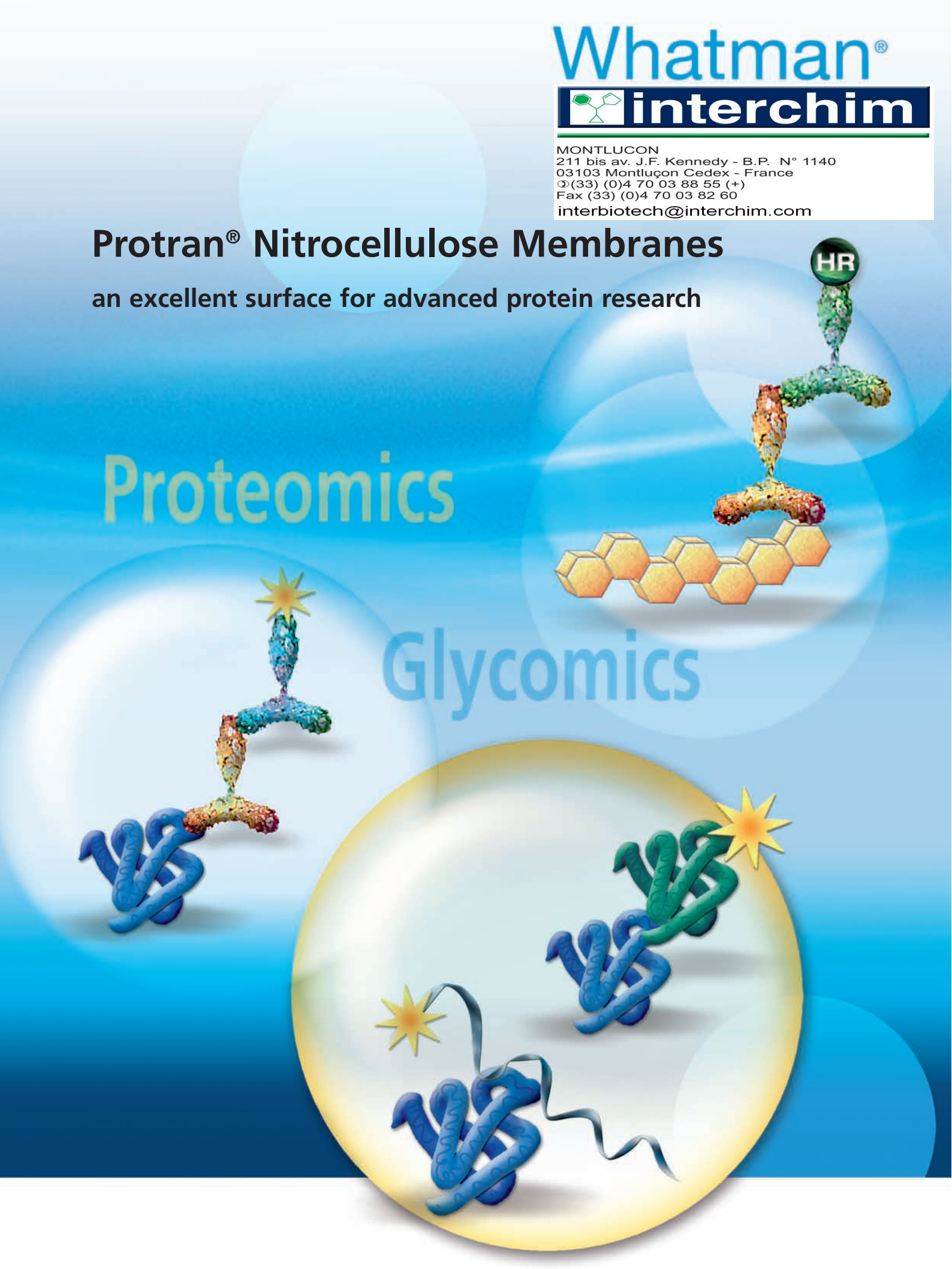
Protran® Nitrocellulose Membranes

an excellent surface for advanced protein research



Protran® is the most frequently specified blotting membrane in the world

Protran nitrocellulose membranes are the most frequently specified transfer media in the world for Western, Southern and Northern blots. All blotting methods have been developed using nitrocellulose membranes and this type of membrane has set the standards.

Nitrocellulose is produced by partial nitration of the natural biopolymer cellulose (Fig. 1). Nitration is an essential prerequisite for production of a microporous membrane that shows all properties of an excellent blotting membrane

Protran nitrocellulose from Whatman is produced from carefully selected and validated raw materials using 100% pure nitrocellulose to ensure highest binding capacity and performance in biomolecule detection.

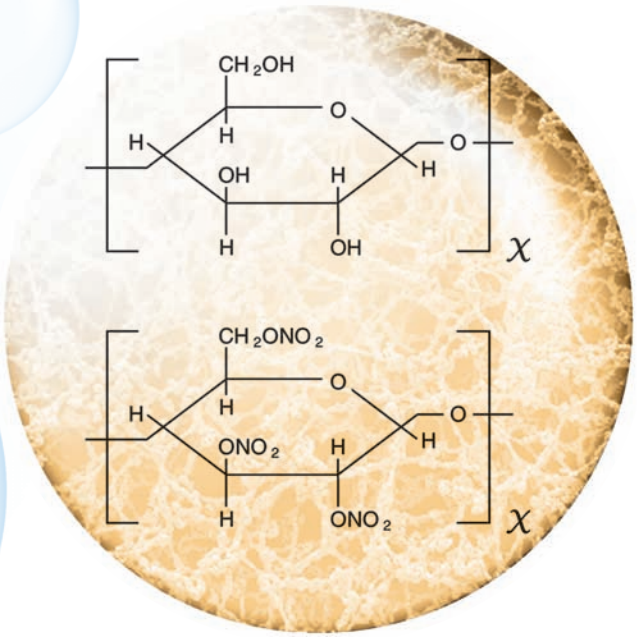


Fig 1: Structure of nitrocellulose

(a) Cellulose, (b) After nitration; Degree of substitution of hydroxyl groups ranges from 1.9 to 2.4.

Protran® shows a superior mechanical strength

Production parameters and the use of proper raw material strongly influence the mechanical properties of a nitrocellulose membrane.

Mechanical stability of a nitrocellulose membrane is of great importance for its performance in the hand of researchers and can be quantified by tensile strength. In this test a defined membrane strip is subjected to a tension of increasing strength.

An investigation of nitrocellulose membranes from all leading manufactures clearly shows that Protran has the highest mechanical stability (i. e., tensile strength) of all nitrocellulose membranes (Fig. 2). In addition, the mechanical strength of Protran is reproducible from lot to lot. This makes Protran nitrocellulose dependable for a wide range of applications.

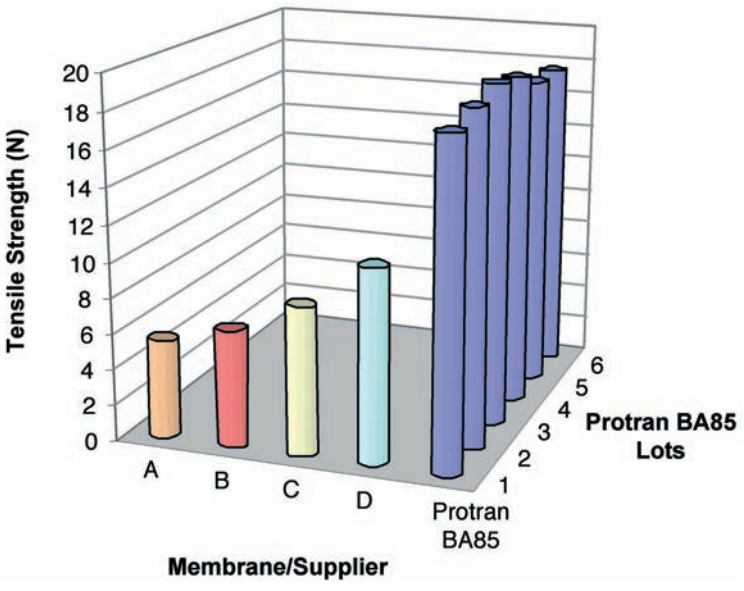


Fig. 2: Mechanical strength of nitrocellulose membranes

Nitrocellulose membranes, 0.45 µm pore size, from different manufactures/suppliers were cut into pieces of 15 x 100 mm. Tensile strength was measured (according to DIN 53 112, part 1) in an automatic tension and pressure recording apparatus. Data indicated are the mean of at least 4 independent measurements. Membrane thickness: A = 130 µm, B = 130 µm, C = 160 µm, D (mixed ester) = 160 µm and Protran BA85 = 125 µm.

In the post-genomic era protein studies have become increasingly more and more important.

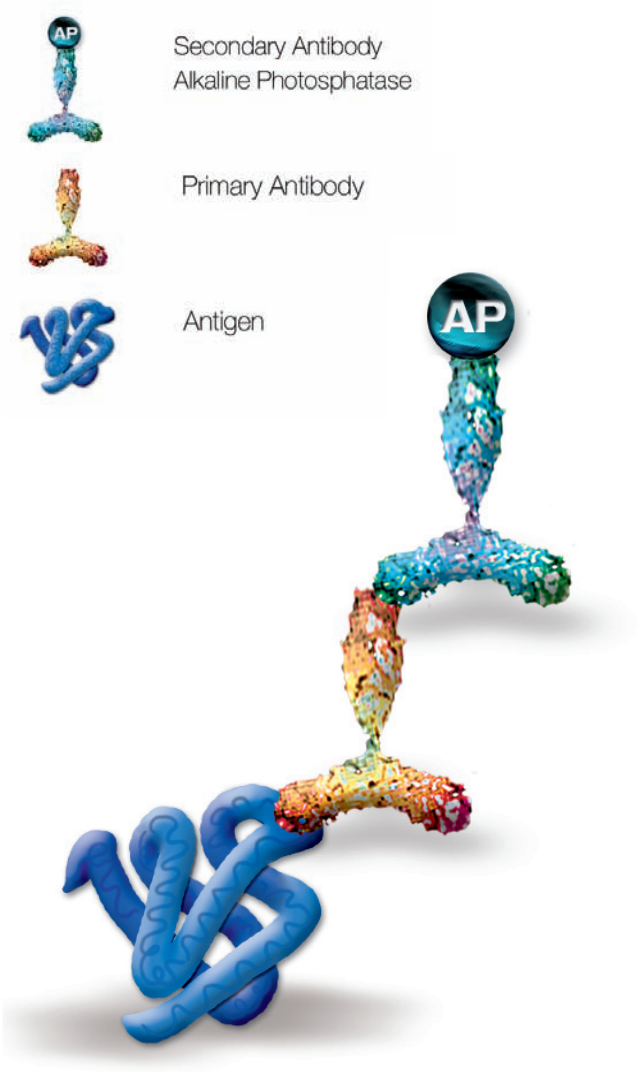
Protran nitrocellulose is a universal blotting surface for biomolecules like nucleic acids and proteins. However, especially for protein applications Protran nitrocellulose has unique properties which make it the best membrane for research.

Protran® affords protein stability for years

A significant advantage of the proprietary Protran nitrocellulose formula is the proven excellent shelf life of proteins applied onto the membrane. Empirical evidence shows that proteins maintain molecular recognition activity for 5 years on Protran (Fig. 3), which makes it the industry standard for protein blotting.

Protran is a small pore size membrane which has been used to produce millions of blots. Whatman also offers a variety of additional large pore size nitrocellulose membranes that have been used with confidence by diagnostic device manufactures for over 20 years.

For example in Point of Care tests like commercial pregnancy test systems a long protein shelf life is an essential prerequisite. For researchers it is an opportunity to improve the shelf life of their blots. Both, for large scale proteomics projects, as well as commercial product development, long-term stability of proteins on Protran nitrocellulose is a unique benefit.



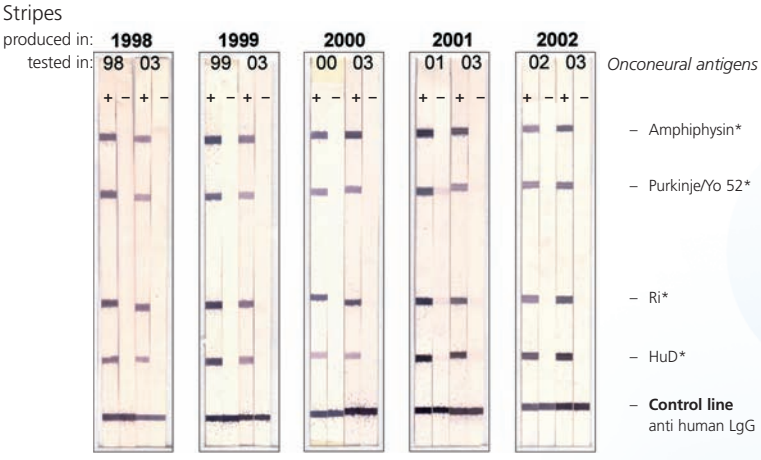


Fig. 3: Stability test of onconeural autoantigens immobilized on nitrocellulose Protran® BA83

Time course was 5 years (1998 to 2002).
Antigens were printed in 20 mM Tris-HCl, 160 mM glycin pH 8.6. Membrane strips were dried, sealed in a plastic pouche with a dessicant pad and stored dark at 2-8 °C. For detection membrane strips were blocked, incubated with human sera and washed. Patient antibodies bound to the antigens were visualized using anti-human IgG antibody conjugated to alkaline phosphatase and p-nitrophenyl phosphate as substrate ("ONA Blot"). The four strips shown in each box respectively originate from the same batch.

+ indicates the result with a mixture of sera reacting with all four onconeural antigens
– indicates the result with a negative serum
* Amphiphysin, Yo 52, Ri, HuD are nuclear proteins present in nerve cells. They can give rise to auto immune diseases with paraneoplastic symptoms.

They can be associated with various cancer types (lung, ovary, breast)
(Data are kindly provided by Prof. Seelig and colleagues, Karlsruhe, Germany, www.laborseelig.de)

Protran® is excellent for carbohydrate studies

Protran nitrocellulose is not only an excellent surface for well-established Western blotting applications. After the advent of genomic and proteomic studies other fields of research are also moving into the focus of interest.

The latest "-omics" suffix describes the study of carbohydrates, called glycomics. Since the decoding of the human genome has revealed no more than 50 000 encoded proteins the importance of post-translational modifications for a precise adjustment of metabolism has been emphasised. Well-known modifications of proteins like glycosylation help proteins to get a proper three-dimensional structure or address them to the right location in the cell.

Recently more and more information is accumulating about proteins that act through oligosaccharide recognition.

One approach for discovering new carbohydrate-recognizing proteins in the proteome, and for mapping carbohydrate recognition structures in the glycome, are arrays of oligo-saccharides [1]. Fig. 4 shows the highly sensitive detection of carbohydrates on Protran nitrocellulose membranes. A quantitative immunostaining experiment clearly indicates a more than 10-fold higher signal intensity for oligosaccharides (as NGLs*) on Protran nitrocellulose.

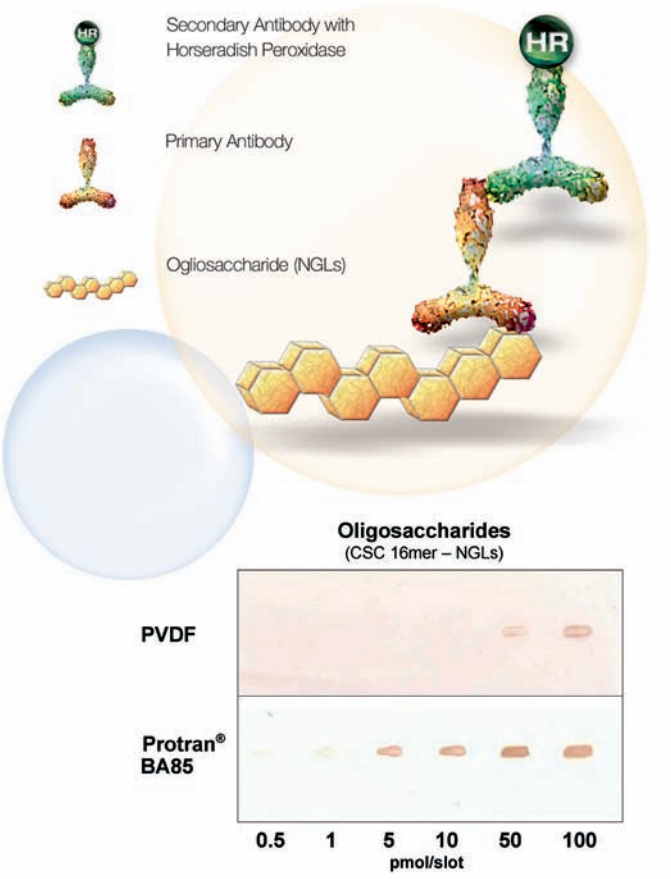
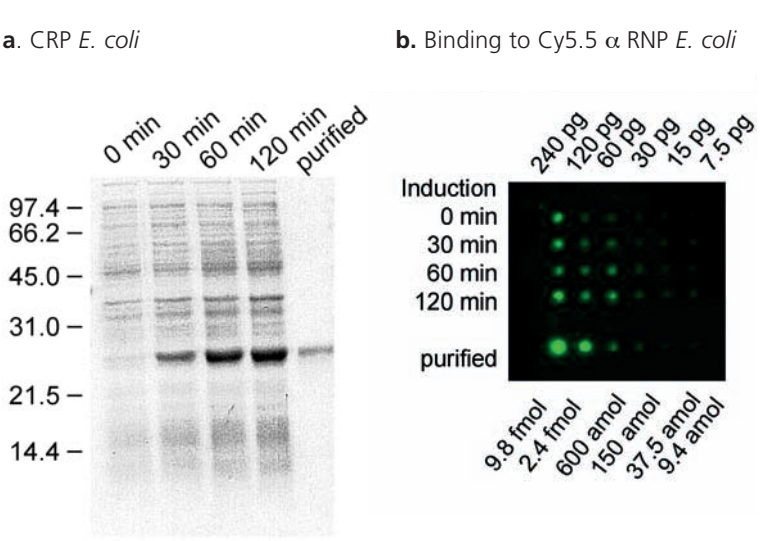


Fig. 4: Immuno detection of oligosaccharides immobilized as NGLs on Protran® BA85

Oligosaccharide NGLs* of acidic 16mer from chondroitin sulfate C (CSC 16mer) were printed in chloroform/methanol/water (25:25:8 vol/vol/vol). For the amounts indicated 2 mm slots were applied onto Protran BA85 nitrocellulose and poly-vinylidene fluoride (PVDF) membranes with a sample applicator. For detection membranes were blocked, incubated with anti-CS (CS-56, mAb) and washed. Antibody binding was visualized using anti-rabbit IgG antibody conjugated to horseradish peroxidase and 3´-diaminobenzidine reagent as substrate ("DAB-FAST"). * Neoglycolipids (NGLs) can be prepared by reductive amination of the reducing oligosaccharide with an amino lipid. The NGL technology generates lipid-linked oligosaccharide probes from glycoproteins and polysaccharides which are particularly suitable for the arraying of oligosaccharides.
(Data are kindly provided by Professor Ten Feizi, Imperial College London, Northwick Park & St Mark's Hospital Campus Harrow, Middlesex, UK.
The method is described in [1].)

Protran® offers highest sensitivity for protein-protein interaction studies

A key role in fine tuning of metabolism is taken by regulatory proteins that act through the recognition of nucleotide or amino acid sequences. Using fluore-scence labelled probes and protein samples spotted on Protran enables analysis of signals from protein-protein (Fig. 5) or protein-DNA interactions (Fig. 6) with high sensitivity of detection.



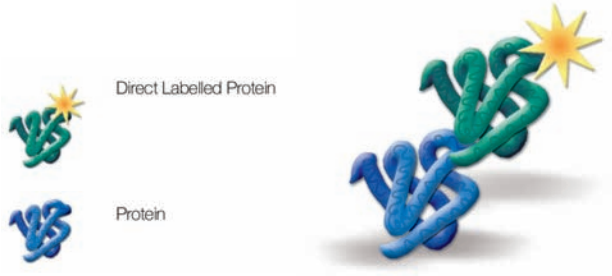
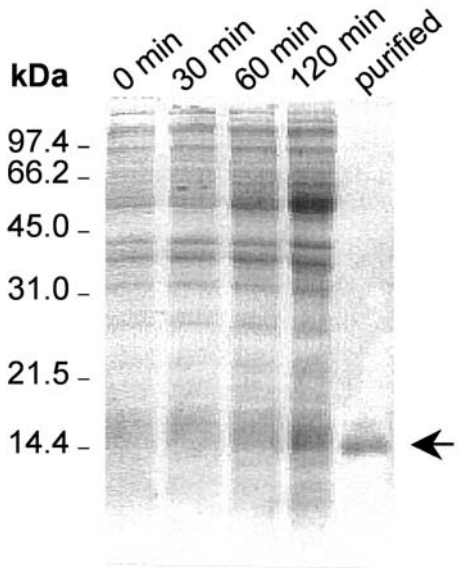


Fig. 5: SDS PAGE analysis and fluorescence detection of protein protein interactions on Protran® BA83

(a) Total protein (crude extracts) of noninduced and 30, 60 and 120 min IPTG induced samples of E. coli CRP (cAMP receptor protein) and purified His-tagged CRP were analysed by polyacrylamide gel-electrophoresis. (b) For the amounts indicated CRP arrays were prepared with the same crude extracts or purified protein. Total protein amount is shown in pg, the amount of purified CRP is shown in fmol and amol. Extracts were spotted on a Protran BA83 nitrocellulose membrane. Binding reactions were carried out in the presence of 5 mM cAMP with a Cy5.5 labelled *E. coli* RNA polymerase a subunit. Protein-protein binding was detected after incubation in PBS solution and with corresponding Cy5.5 labelled protein with The Odyssey Infrared Imaging system (LI-COR).
(Data see under Fig. 6)

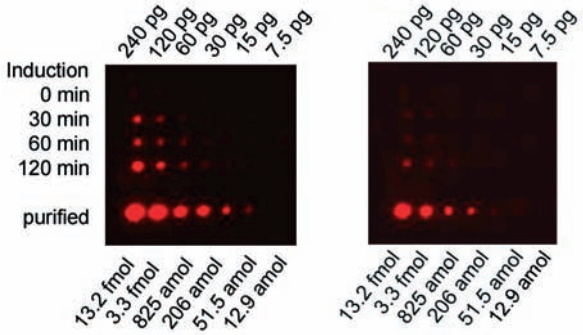
a. ArgR *B. stearothermophilus*



Binding to IRD-800

b. 76 bp DNA

c. 56 bp DNA



In a protein-protein binding assay *E. coli* cyclic adenosine monophosphate receptor protein (CRP) was printed on Protran nitrocellulose and incubated with Cy5.5 labelled *E. coli* RNA polymerase α subunit (α RNP). It was possible to detect a signal from a purified *E. coli* CRP spot with 150 amol protein (Fig. 5b).

In a protein-DNA binding assay up to 12 amol of a spotted protein could be detected using IRD-800 labelled DNA probes. Serial purified Arginine repressor (ArgR) protein were spotted on Protran nitrocellulose and incubated with two different shortened derivates of a PargCo promoter-operator region (Fig. 6 b, c). linear relationship of fluorescent response was measurable over more than 2 orders of magnitude (between 3.3 fmol and 12.9 amol of purified ArgR protein). Even the higher affinity of the tested repressor protein to a double Arg box-carrying DNA probe (Fig. 6 b) compared to single Arg box-carrying probe (Fig. 6 c) was detectable.

The data shown document the outstanding features of Protran nitrocellulose membranes in molecular research. The advantage of biomolecules immobilised on nitrocellulose is obvious. Non-covalent immobilisation on nitrocellulose avoids the modi fication of biomolecules like in approaches with covalent binding on chemically activated surfaces and preserves molecular recognition activity. Altogether the mechanical strength of Protran as well as long-term stability and accessibility of proteins for binding reactions are of great importance for a stable and sensitive assay and reliable results.

"Concerning the choice of NC brand we have found Schleicher & Schuell's* to be the best (highest binding capacity, highest signal:background ratio)..." [3]. – *[now Whatman]

What was true when the first blot on nitrocellulose was carried out is still highy accurate and important today.

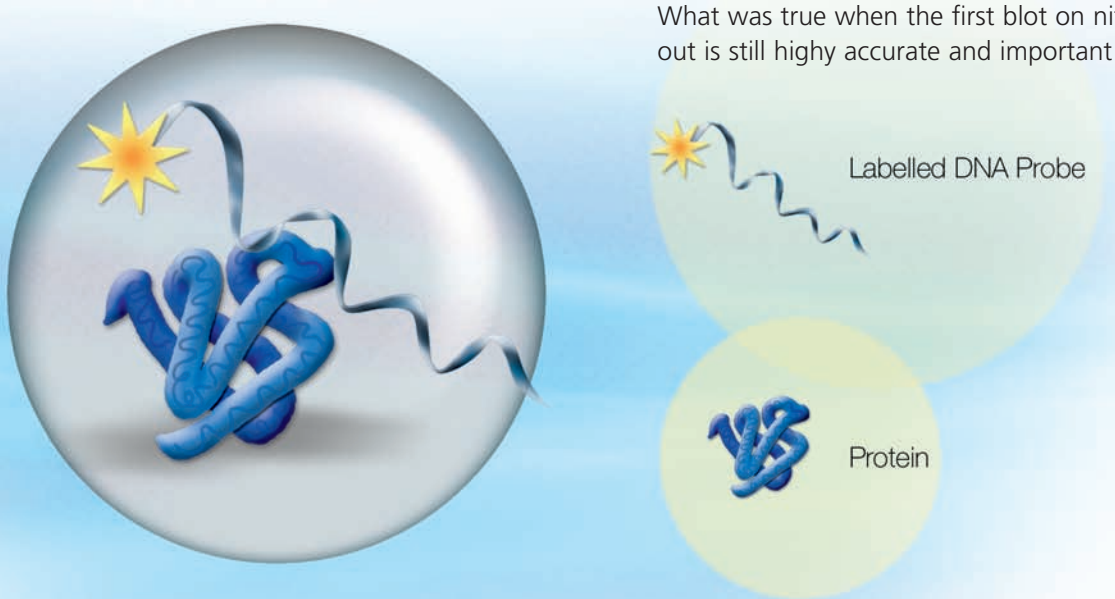


Fig. 6: SDS PAGE analysis and fluorescence detection of protein-DNA interactions on Protran® BA83

(a) Total protein (crude extracts) of noninduced and 30, 60 and 120 min IPTG induced samples of B. stearothermophilus ArgR (arginine repressor) and purified His-tagged ArgR were analysed by polyacrylamide gel-electrophoresis. (b, c) For the amount indicated ArgR arrays were prepared with the same crude extracts or purified protein. Total protein amount is shown in pg, the amount of purified ArgR is shown in fmol and amol. Extracts were spotted on a Protran BA83 nitrocellulose membrane. Binding reactions were carried out with a 76 bp IRD-800 labelled DNA probe of the entire B. stearothermophilus PargCo region (containing a double Arg-box carrying operator) or with a 56 bp IRD-800 labelled DNA probe of a shortened PargCo region (containing a single Arg-box carrying operator). DNA-protein binding was detected after incubation in DNA-binding buffer and IRDye 800-labelled DNA probe with the Odysses Infrared Imaging system (LI-COR).

Fig. 5 and 6: (Data are kindly provided by Professor Vehary Sakanyan, ProtNeteomix & Nantes University, Nantes, France, www.protneteomix.com. The method is described in [2].)

SPECIFICATIONS

Protran® 100 % pure nitrocellulose

APPLICATIONS	*Western, Southern, Northern blotting...*
BINDING CAPACITY	
* pore size: 0.45 µm	80 µg/cm²
* pore size: 0.2 µm	90 µg/cm²
* pore size: 0.1 µm	110 µg/cm²
TRANSFER METHODS:	
Semi-dry blotting	++
Tank blotting	++
Vacuum blotting	++
Capillary blotting	++
Alkaline method	not recommended
IMMOBILIZATION:	
Drying	Proteins
UV-crosslinking	DNA/RNA
Baking (80°C)	DNA/RNA
DETECTION METHODS:	
Colorimetric	++
** Chemiluminescent	++
Isotopic	++
Fluorescent	Proteins

* Protran membranes are available in a range of pore sizes for optimal performance in a variety of applications. 0.45 µm pore size Protran BA85 is the general lab standard for most protein and nucleic acid applications. The 0.2 µm pore size of Protran BA83 ensures high retention of small samples below 20 kD. BA79 is the membrane of choice for smaller molecules below 7 kD.

** Protran Nitrocellulose Membranes are compatible to a variety of detection methods. In case of chemiluminescent detection we recommend horseradish peroxidase based systems.

Find out more about the best platform for protein microarrays:
www.arraying.com

ORDERING INFORMATION

Description	Pore Size (µm)	Size (mm)	Qty / Pkg	Item #
Protran BA85, circles	0.45 µm	ø 82	50	10401116
"	0.45 µm	ø 132	25	10401124
Protran BA85, sheets	0.45 µm	200 x 200	5	10402680
"	0.45 µm	200 x 200	25	10401191
"	0.45 µm	300 x 600	5	10401180
Protran BA85, roll	0.45 µm	200 x 3 m	1	10401197
"	0.45 µm	300 x 3 m	1	10401196
Protran BA83, circles	0.2 µm	ø 82	50	10401316
Protran BA83, sheets	0.2 µm	200 x 200	5	10402452
"	0.2 µm	200 x 200	25	10401391
"	0.2 µm	300 x 600	5	10401380
Protran BA83, roll	0.2 µm	200 x 3 m	1	10402495
"	0.2 µm	300 x 3 m	1	10401396
Protran BA79, sheets	0.1 µm	200 x 200	5	10402062
"	0.1 µm	200 x 200	25	10402091
"	0.1 µm	300 x 600	5	10402080
Protran BA79, roll	0.1 µm	300 x 3 m	1	10402096

Accessory Products	Thickness	Size (mm)	Qty / Pkg	Item #
Blotting Paper 3MM, sheets	0.34 mm	200 x 200	100	3030-801
"	0.34 mm	460 x 570	100	3030-917
Blotting Paper 17 Chr, sheets	0.92 mm	460 x 570	25	3017-915
Blottingpaper GB004, sheets	1.00 mm	460 x 570	100	10427926
Blottingpaper GB005, sheets	1.50 mm	580 x 580	25	10426994

Other formats are available upon request!

Whatman offers the complete range of blotting membranes! Optitran™, reinforced nitrocellulose membrane, Nytran™ nylon and Westran™ PVDF membranes are high performance membranes for nucleic acid and protein research.

REFERENCES

[1] Fukui S., Feizi T., Galustian C., Lawson A. M. and W. Chai. Oligosaccharide microarrays for high-throughput detection and specificity assignments of carbohydrate-protein interactions. Nature Biotechnology 2002, Vol. 20, 1011-1017

[2] Snapyan M., Lecocq M., Guźel L., Ar naud M.-C., Ghochikyan A., and V. Sakanyan. Dissecting DNA-protein and protein-protein interactions involved in bacterial transcriptional regulation by a sensitive protein array method combining a near-infrared fluorzescence detection. Proteomics 2003, 3, 647-657.

[3] Bjerrum O. J. and N. H. H. Heegaard (eds.). CRC Handbook of Immunoblotting of Proteins. CRC Press, Boca Raton, Florida, Vol. 1, 105.

FURTHER INFORMATION

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