



Agilent 3100 OFFGEL Fractionator

pl-based fractionation of
proteins and peptides
with liquid-phase recovery



Agilent Technologies

Achieve unprecedented sensitivity in LC/MS-based proteomics experiments



Sample complexity and large dynamic range of protein and peptide concentrations are major hurdles in the analysis of low abundance proteins. Today, for almost any proteomic analysis, robust and reproducible fractionation techniques are key to ensure that MS/MS can identify the maximum number of proteins. At the same time, to avoid inadvertent loss or alteration of critical proteins, it is important that fractionation steps minimize the number of sample manipulations.

OFFGEL electrophoresis is a novel technique jointly developed by Agilent Technologies and Diagnoswiss* that separates proteins or peptides according to their isoelectric points, whereby the separated components are recovered in liquid phase. Up- or downstream sample processing steps such as immunodepletion, protein digestion and liquid chromatography can be easily interfaced with this technique for multi-dimensional separations of complex samples.

“The instrument is highly user-friendly and simple to use. In our group, the scientists can operate the OFFGEL system after just an hour of training”

Hookeun Lee, Senior Scientist at ETH, Zurich

“We had the opportunity to test the system prior to market release and have found that high resolution pI prefractionation allowed us to increase our sensitivity in analysis of complex samples”

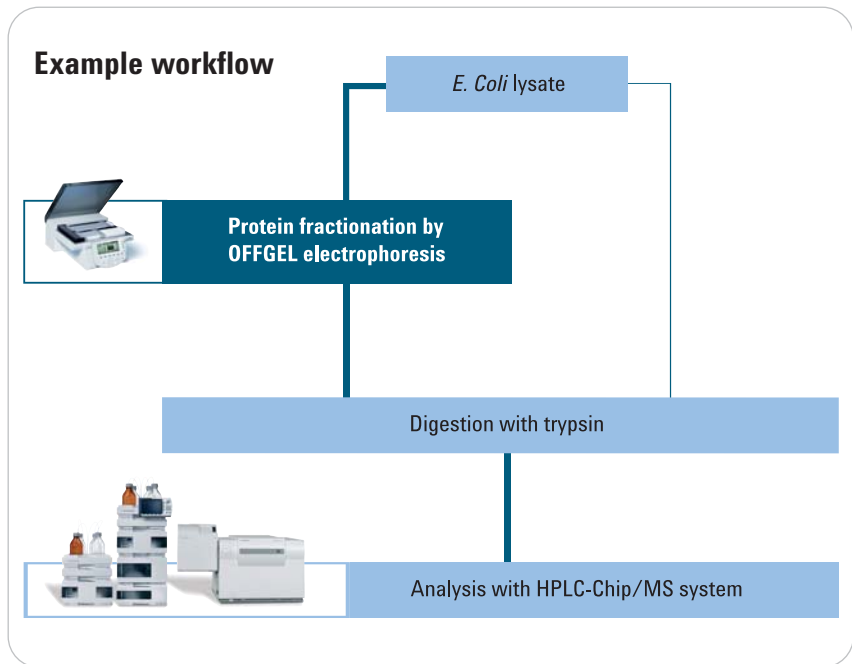
Edward Dratz, Montana State University

Easy integration into any protein analysis workflow

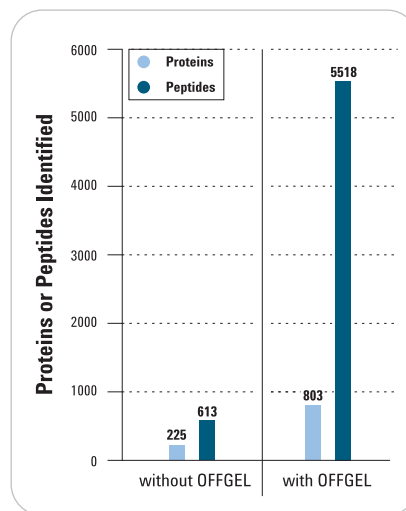
Whether your task is biomarker discovery, protein identification or purification of functional proteins or peptides, the Agilent 3100 OFFGEL Fractionator fits into any protein analysis workflow. Integration of the OFFGEL system is easy and ultimately increases sensitivity of subsequent MS detection.

Benefits of the Agilent 3100 OFFGEL Fractionator

- pI-based OFFGEL fractionation with liquid-phase recovery for easy transfer to downstream techniques such as LC/MS
- Reproducible fractionation with resolution as low as 0.1 pH for maximum MS sensitivity
- Compatibility with up- or downstream techniques such as immunodepletion, LC/MS or gel-based analysis for maximum flexibility
- pI values obtained act as additional validation parameters of MS hits and can be used to search for peptides with charged posttranslational modifications (PTMs)
- All additives can easily be removed after fractionation to avoid interference with nano-electrospray and MS detection
- Fractionation of up to 16 samples in parallel on two trays (eight samples each) for highest throughput
- Broad loading capacity from 50 µg to 5 mg of sample for analytical applications or maximum enrichment of low abundance proteins
- OFFGEL mode as well as conventional isoelectric focusing (IEF) based on immobilized pH gradient (IPG) gels

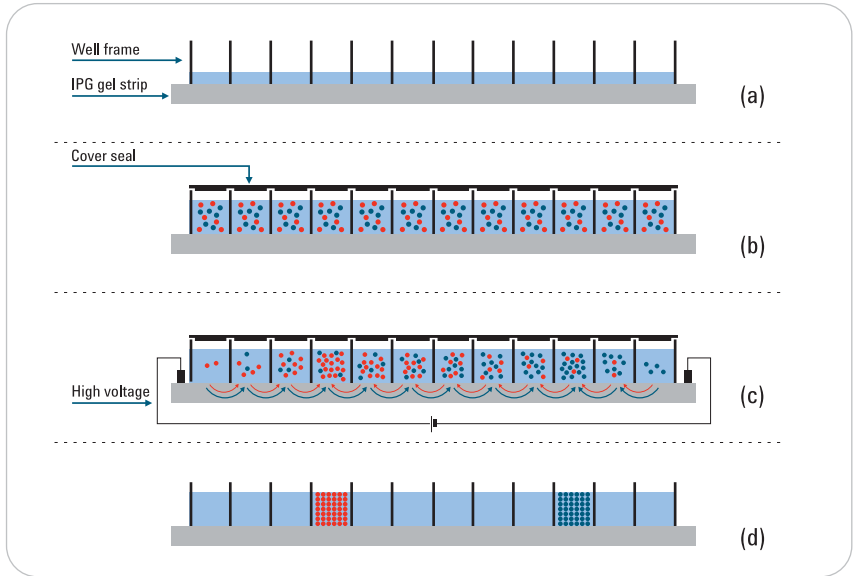


Adding OFFGEL fractionation into the workflow prior to digestion significantly increases the number of proteins and peptides identified by subsequent HPLC-Chip/MS analysis.



HPLC-Chip/MS analysis of *E. coli* lysate, showing total number of non-redundant proteins and peptides identified with and without OFFGEL fractionation (pH 4-7, 24 fractions).

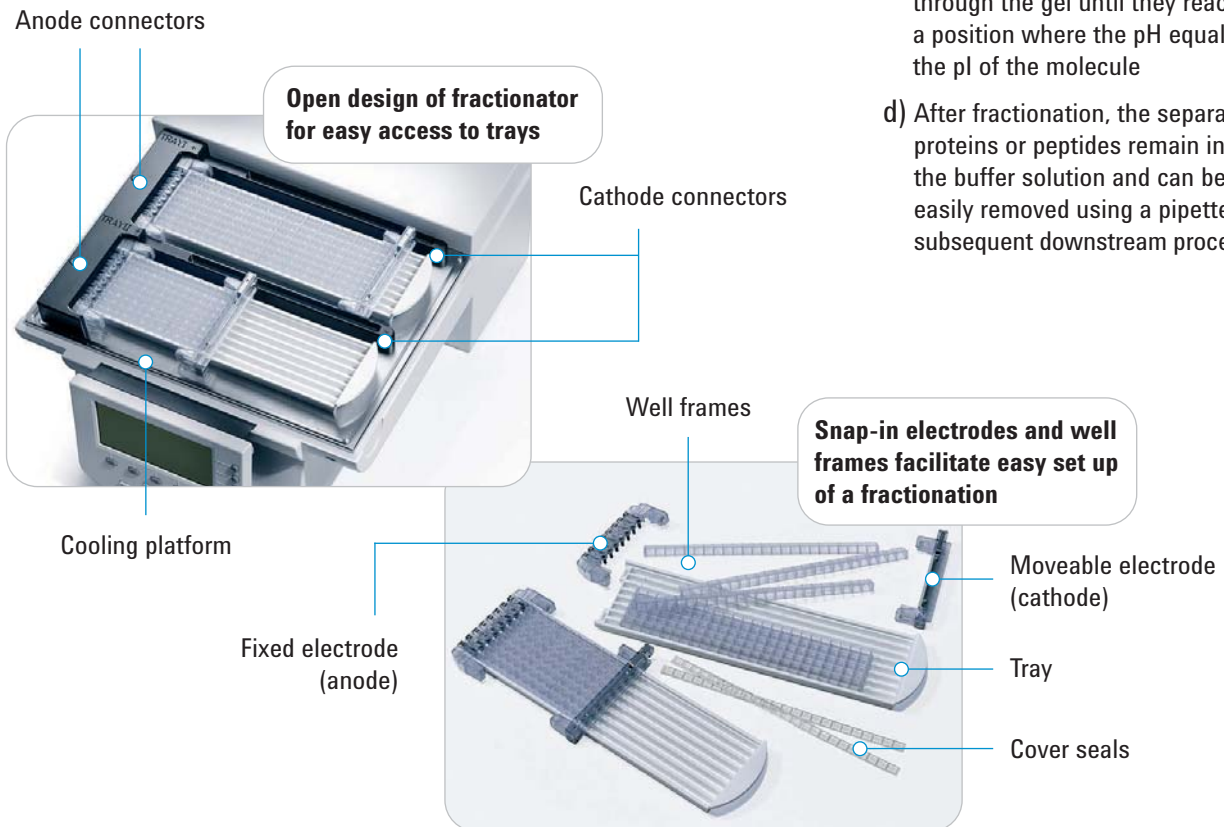
OFFGEL electrophoresis – high resolution pI fractionation based on proven IPG technology



Fractionation principle

The Agilent 3100 OFFGEL Fractionator performs isoelectric focusing of proteins or peptides in immobilized pH gradient (IPG) gel strips. The technique applied by the 3100 OFFGEL Fractionator differs from conventional gel electrophoresis in that the sample components are recovered from the liquid phase. This makes the technology directly compatible with liquid-phase workflows, for example LC-MS.

- a) The IPG gel strip is rehydrated and seals tightly against the well frame
- b) The diluted sample is equally distributed into all wells in the frame, and a cover seal is placed on top of the frame to prevent sample evaporation
- c) A high voltage is applied to the ends of the gel strip and the protein or peptide molecules migrate through the gel until they reach a position where the pH equals the pI of the molecule
- d) After fractionation, the separated proteins or peptides remain in the buffer solution and can be easily removed using a pipette for subsequent downstream processing



Individual sample diagnostics for a quick and easy check of fractionation quality

The 3100 OFFGEL Fractionator measures and records voltage, power and current during fractionation in OFFGEL and traditional IEF mode. The current for each sample is measured individually. The current/voltage information can be visualized using the controller's online plot or by exporting the data to a spreadsheet application, and provides a diagnostic indicator for the quality of each individual sample fractionation.

Tray I		0.077 kWh	OG12PR00	Ready	10:15:38																
Focusing		0.03 hzm		Current																	
Limit	Actual	<table border="1"> <tr><td>A</td><td>0.0</td></tr> <tr><td>B</td><td>0.0</td></tr> <tr><td>C</td><td>0.0</td></tr> <tr><td>D</td><td>0.0</td></tr> <tr><td>E</td><td>0.0</td></tr> <tr><td>F</td><td>0.0</td></tr> <tr><td>G</td><td>0.0</td></tr> <tr><td>H</td><td>0.0</td></tr> </table>				A	0.0	B	0.0	C	0.0	D	0.0	E	0.0	F	0.0	G	0.0	H	0.0
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VoltHours	20	0.076 kWh	0.0 [µA]																		
Voltage	8000	1333 V	50.0																		
Power	200	66.7 mW																			
Current	50.0	50.0 µA																			

Typical current pattern on tray I when every second lane is running an OFFGEL fractionation.

Features of the Agilent 3100 OFFGEL Fractionator

- Easy setup with preinstalled methods for OFFGEL and conventional IPG-based IEF modes
- Independent high-voltage power supplies allow two different methods or sample sets to be run simultaneously
- Online display of all important fractionation parameters and online current measurement of each individual sample for monitoring of key parameters that affect fractionation quality
- Automated storage of up to 1200 data files and easy export of run parameters
- Monitoring of all operations and error events in self-updating logbook
- Cover design allows fractionation of light-sensitive protein samples

Five easy steps to set up and run a fractionation



1. Place a dry IPG gel strip in the tray.



2. Place a well frame over the IPG gel strip, pipette 20 µl rehydration solution into each well and allow the IPG gel to swell.



3. Pipette 150 µl of the diluted sample into each well and close the frame with a cover seal.



4. Attach the electrodes to the tray.

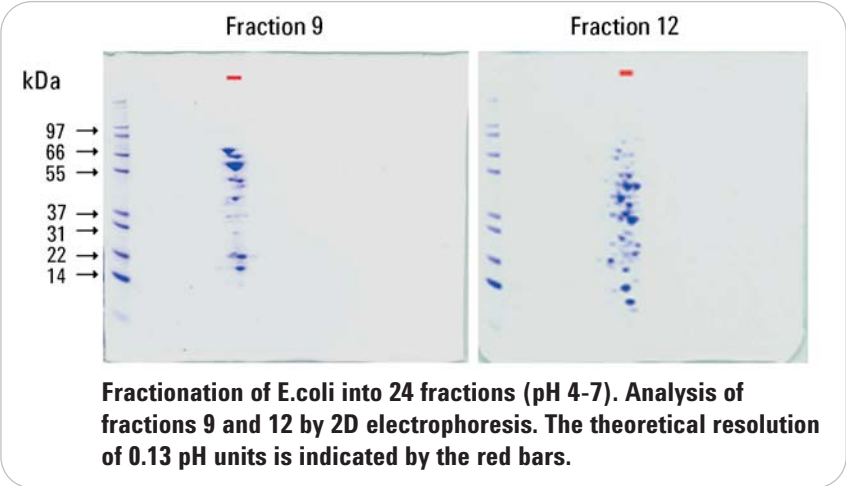


5. Place the loaded tray into the fractionator and press "Start".

Protein Fractionation

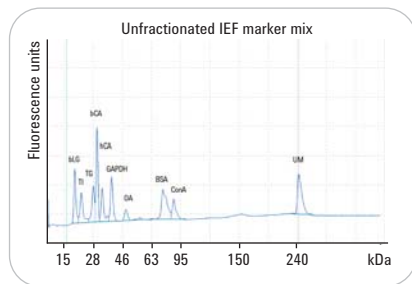
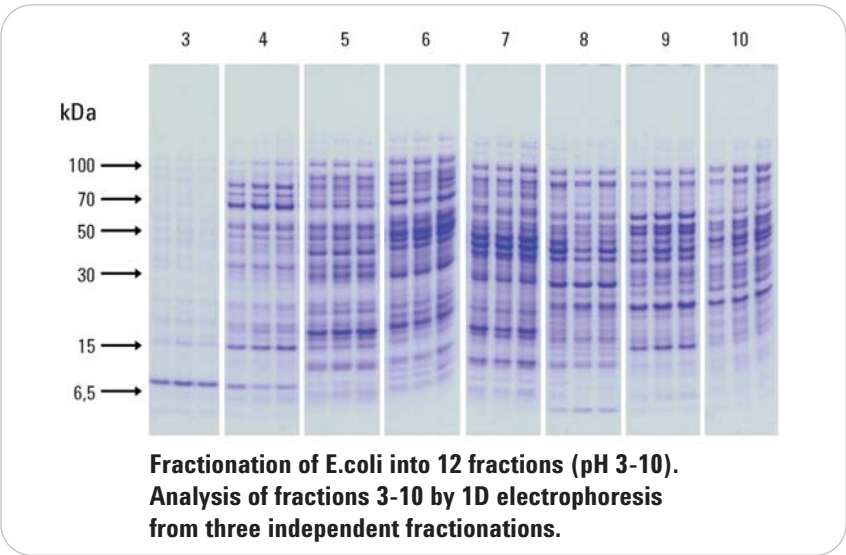
Achieve highest resolution

The Agilent 3100 OFFGEL Fractionator provides pI-based fractionation of peptides or proteins with exceptional resolution. Samples can be fractionated into 12 or 24 fractions for standard or highest resolution.

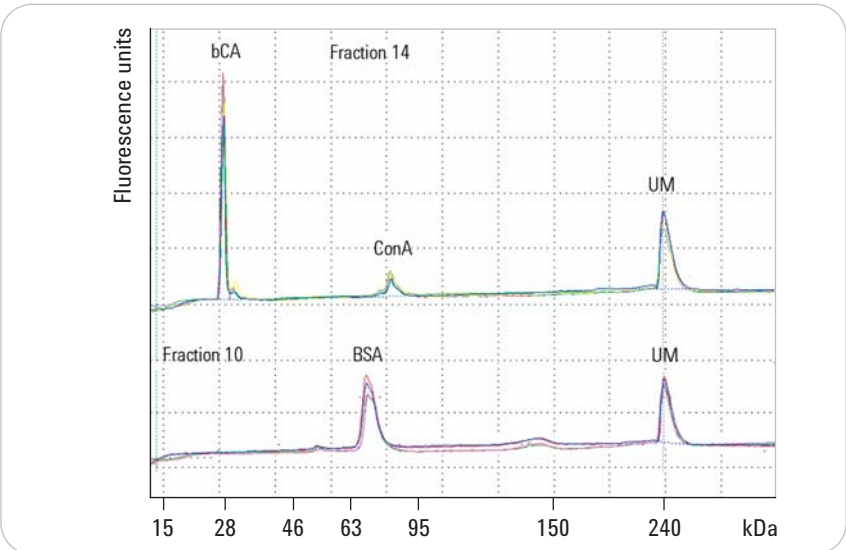


Obtain greater reproducibility

Fractionation by OFFGEL electrophoresis is highly reproducible. Fractions from several runs may be pooled for enrichment of low abundance proteins or peptides.



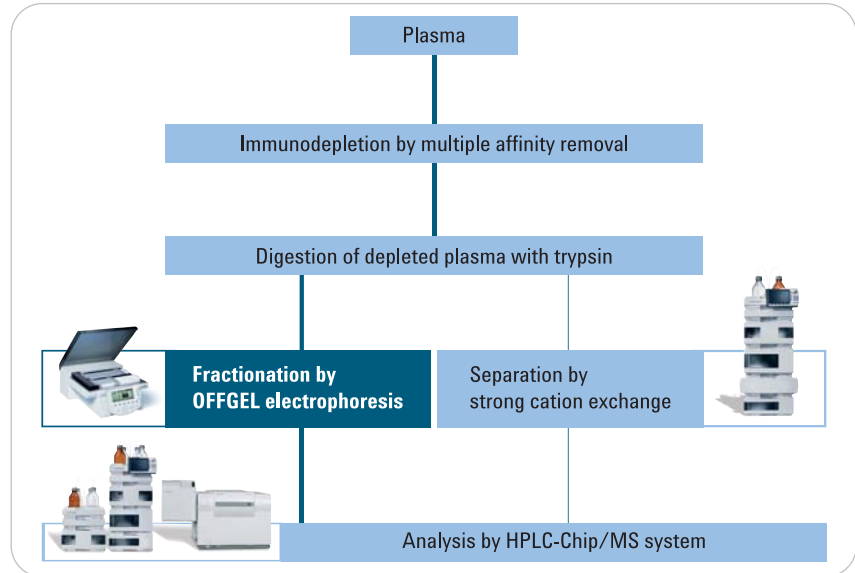
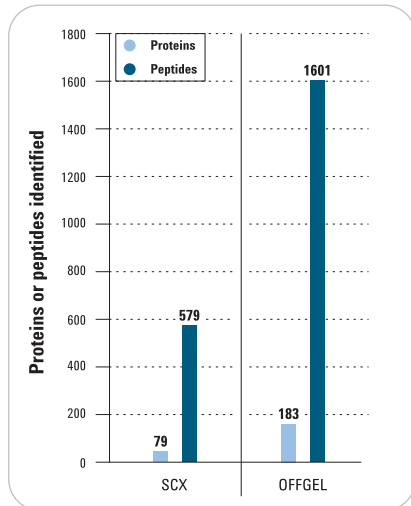
Analysis of a protein IEF marker mix with the Agilent 2100 bioanalyzer before and after OFFGEL electrophoresis (24 fractions, pH 3-10). The overlay of electropherograms from five independent fractionations shows excellent reproducibility (UM - upper marker, bCA - bovine carbonic anhydrase II, BSA - bovine serum albumine, ConA - conalbumine).



Peptide Fractionation

Gain sensitivity

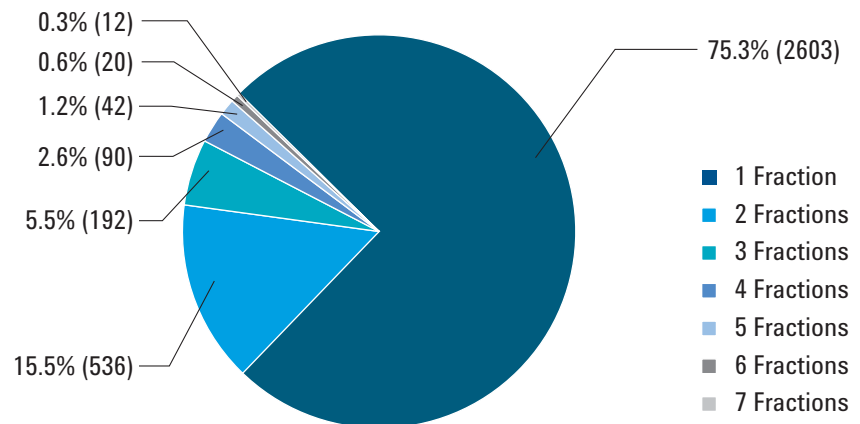
Efficient reduction of sample complexity increases sensitivity of downstream analysis. This example compares the efficiency of fractionation of the tryptic peptides of immunodepleted plasma by strong cation exchange (SCX) with OFFGEL electrophoresis.



Peptide fractionation by OFFGEL electrophoresis (pH 3-10, 24 fractions) is more efficient than separation by SCX (50 fractions) as indicated by the 3-fold higher number of peptides and 2-fold higher number of proteins identified with HPLC-Chip/MS.

Increase efficiency

Whereas proteins are often represented by isoforms with different pI values, peptides have distinct, individual pI values. In this example, 90 % of all individual tryptic peptides focused efficiently into one or two OFFGEL fractions.



Number of OFFGEL fractions containing each individual peptide (absolute numbers of peptides in parenthesis).

Agilent 3100 OFFGEL Fractionator



Instrument specifications

Type	Specification	Comments
Weight	14 kg (31 lbs)	
Dimensions (height × width × depth)	157 × 355 × 427 mm (6.2 × 14 × 16.8 inches)	
Line voltage	100 – 240 VAC, ±10%	Wide ranging capability
Line frequency	50 – 60 Hz, ±5%	
Power consumption	140 VA	
Ambient operating temperature	5 – 40 °C	(41 – 104 °F)
Humidity	< 80% at 40° C (104 °F)	Non-condensing
Operating altitude	Up to 2000 m (6500 ft)	
Safety standards IEC, CSA, UL	Installation Category II, Pollution Degree 2	
Flash memory card	minimum 512 MB storage capacity	
Platform temperature	10 °C – 35 °C	Maximum 10 degrees below ambient, non-condensing
Power supplies	Two independent high voltage power supplies Voltage-Range: 0.5 – 10 kV Current-Range: 0 – 150 µA/strip Power: 0 – 1 W/strip Modes: Constant voltage Constant current Constant power Timetable programable	Individual current measurement for each sample
Fractionation capacity	2 × 8 samples (12 or 24 fractions) in OFFGEL mode and IPG-IEF (in-gel) on 2 individual trays	

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© Copyright 2006 Agilent Technologies
Published March 1, 2007
Publication Number 5989-5700EN



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