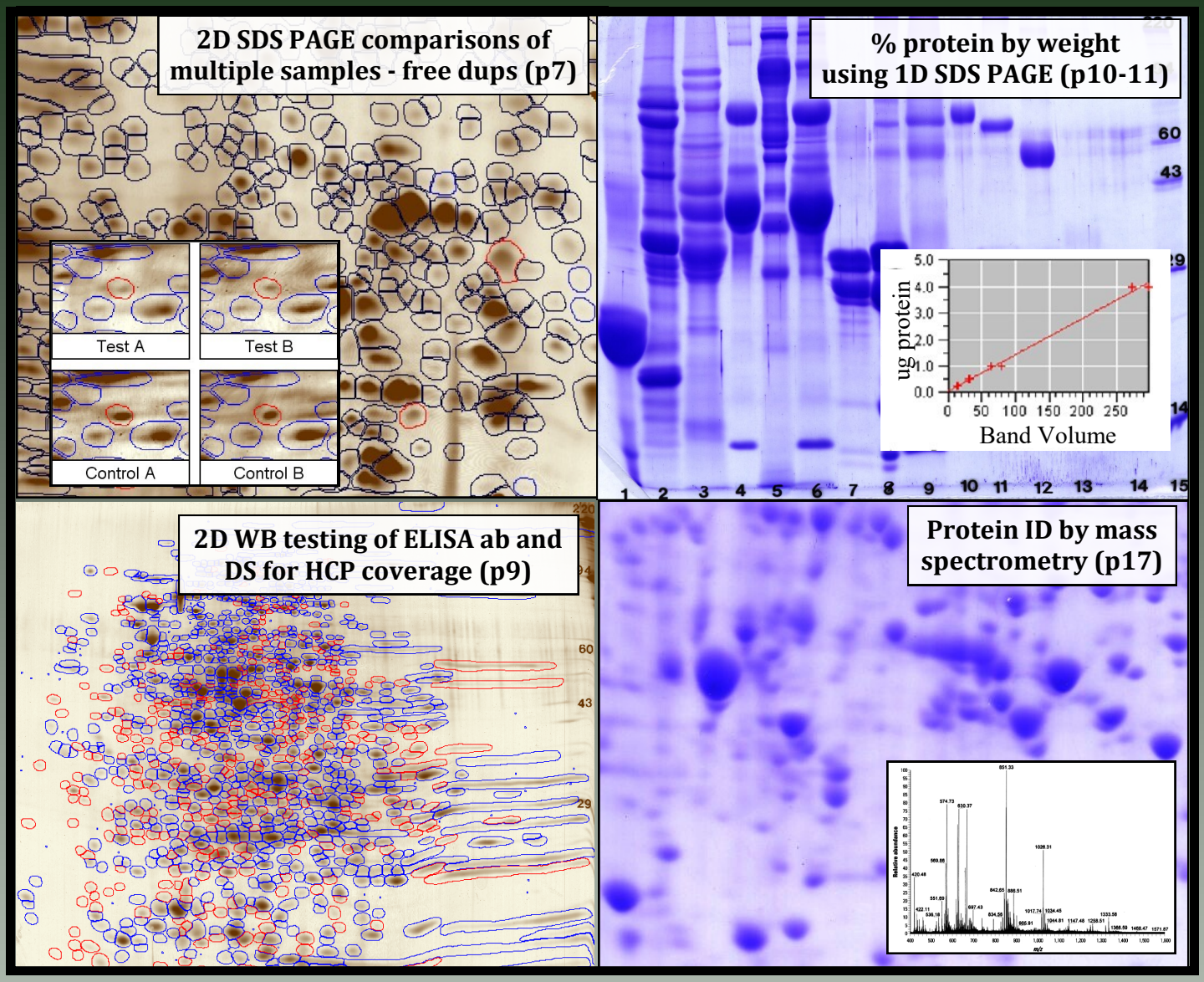

Kendrick Labs, Inc.

CONTRACT PROTEIN ANALYSIS

2025 PRICE GUIDE

Send samples to: Kendrick Labs Inc, 1202 Ann St, Madison, WI 53713
800-462-3417 (local 608-258-1565) Email: 2d@kendricklabs.com

kendricklabs.com

January 2025

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How our protein analysis service works:

You ship samples to us on dry ice, and our expert technical staff does the work. Images of 2D gels and blots are emailed to you for approval after about a week, depending on treatment. If something goes awry, we do free repeats until you're satisfied. Once gel images are approved, custom quantitative analysis including a complete report is performed. GLP/GMP projects are welcome. Kendrick Labs is registered with the FDA and performs all tests according to written SOPs using validated equipment. We are available at any point via email or phone (1-800-462-3417) to discuss your project and provide a price quote.

Supplemental Services:

- **Computer Comparisons** include scanning your 2D gels with a calibrated densitometer, matching and quantifying the patterns using sophisticated SpotMap software from TotalLab, Ltd, and presenting results in a complete report.
- **Staining** techniques include high sensitivity silver staining as well as Coomassie blue and fluorescent stains along with appropriate densitometry.
- **Western blotting** procedures include transblotting and immunostaining with the antibody of your choice including ultrasensitive ECL detection.
- **Protein Identification by Mass Spectrometry** (nano-LC/MS/MS) will be arranged as needed.

1DE: A complete line of one-dimensional electrophoresis using SDS slab gels is offered along with quantitative analysis of the bands. Results can be expressed as band percent of total in a lane, or as protein percent by weight based on a standard curve.

Sample Preparation can be done here or there

We're glad to prepare samples here (cell pellets, chunks of tissue etc) for a fee. If you want to do it there and need advice, an instruction booklet is available plus our Lab Manager (Jon Johansen) or Western Blot Manager (Matt Hoelter) will answer questions by phone or email. A 1-step buffer addition will be performed without charge if the samples are ready for dilution. A mailing kit containing premade buffers is a time-saver if you're in a hurry. (**MK-1**; $406 includes shipping)

Rapid Return For gels/blots without analysis, stained, dried gels and PVDF membranes are returned within 4-10 days by Express mail. In Madison, samples are picked up and delivered for free.

Standardization and documentation

- Six molecular weight standards are labeled on every stained 2D gel along with one (or more if requested) IEF internal standard.
- A pH gradient plot, obtained with a pH surface electrode on blank IEF tubes, is included for each set of gels.
- A written summary describing conditions of electrophoresis is provided for every set of samples.
- Full reports are provided as needed with optional run documentation including detailed IEF, slab gel, and western blot checksheets.

Off site Archival of electronic data

Indefinite archival of reports and data files on a password protected website, Egnyte, is included with Run Documentation for clients ordering HCP antibody or DS analyses (item GTRK-1 p9). Other reports and images are immediately emailed to clients and archived on in-house computers backed up by Carbonite. Printed reports, dried gels, western blot films etc. are sent to clients by 2-day express mail.

cGMP-1 Current Good Manufacturing Practice

Numerous SOPs required for cGMP for 1D and 2D electrophoresis are in place at Kendrick Labs along with an archival system for equipment calibrations and reports. However, custom validation for the sample type of interest including SOPs are required for new projects to pass detailed FDA inspections - which may occur years down the road. *Please let us know if you are going to submit an IND, or plan to use our lab for QA of a product.*

Our Guarantee: When you send samples to Kendrick Labs, you'll avoid the myriad of specialized details inherent in this technique. You'll receive fully documented gels, blots and/or reports as quickly as possible by email and/or Express mail. If you're not happy, just call. We'll do our best to make your project successful including free reruns if necessary. If there is a slip-up, either on our end or yours, we'll rerun gels immediately so you won't be left empty-handed. We're on your side.

How to Mail Samples on Dry Ice

Most protein samples must be mailed on either ice or dry ice. Lyophilized samples can be sent at room temperature. Call ahead to discuss, if you're unsure (800-462-3417).

1. Aliquot samples into 1 ml Eppendorf tubes or other small tubes.
2. Label each tube with an identifying name or number with indelible ink. Fill out the sample ID form including tube names and numbers.
3. Freeze the tubes, place in a small box or plastic bag and position on ice packs or dry ice in the shipping box.
4. Put the completed sample ID form inside the shipping box. If appropriate, make sure you have indicated how samples are to be diluted. We encourage as much detail as possible. Additional information often helps in optimization of loads to give better gels. *All information concerning samples is held strictly confidential.*
5. Mail using an overnight service such as FedEx or UPS. If appropriate, check "dry ice" and apply the "dry ice" sticker with number of pounds to outside of package.

Pickup and delivery in the Madison area is free. Call (608-258-1565) to arrange a time.

Kendrick Labs Services & Prices Guide

Call (800-462-3417) or email (2d@kendricklabs.com) to discuss your project or for a price quote.

2-Dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (2D SDS PAGE) is a multistep electrophoretic process for resolving complex mixtures of proteins in biological samples. The samples are first dissolved in either SDS or urea buffer, or a combination of both. While other core labs use commercial immobilized pH gradient strips for isoelectric focusing, Kendrick Labs uses the carrier ampholine/tube gel method because of compatibility with sodium dodecyl sulfate (SDS) as explained on our web site. SDS is the best known reagent for solubilizing proteins.

1st Dimension: Samples are applied to the top of thin acrylamide tube gels polymerized with ampholines. Proteins are separated in the tube gels according to their isoelectric point by the process of isoelectric focusing.

2nd Dimension: After a brief equilibration in SDS buffer, each tube gel is sealed to the top of a stacking gel and SDS slab gel electrophoresis is carried out to separate proteins by their molecular weight on either a Standard Format (SF, 13x15 cm) or Large Format (LF, 20x20 cm) gel. Staining, drying, scanning and analysis follow.

As many as 2100 polypeptide spots may be resolved on a 2D gel as opposed to the 50 band maximum obtained by either dimension alone. 2DE provides unmatched resolution of complex protein mixtures in a way that allows identification of subtle differences between the patterns.

2D Electrophoresis

MK-1 Sample Buffer Mailing Kit

This kit contains 10 one ml tubes of Urea Sample Buffer; 10 one ml tubes of SDS Boiling Buffer; 17 one ml tubes of SDS Boiling Buffer minus BME; 17 one ml tubes of Osmotic Lysis Buffer; 17 100 µl tubes of 10X Nuclease Stock; four 100 µl tubes of Protease Inhibitor Stock Solutions and three 100 µl tubes each of Phosphatase Inhibitor Stock Solutions I and II. The compositions of these solutions is given in the Kendrick Labs' "Suggestions for Sample Preparation" booklet included in the mailing kit. Instructions and materials for mailing are also included.

Price $406 *includes shipping on dry ice*

CON-1 Consulting Fees

Price: $242/hr

2D-ES-1 2D Electrophoresis Basic Service Standard Format Our SF gel is perfect for immunoprecipitations, subcellular fractionations, most bacterial preparations, and many proteomics applications. One Coomassie blue stained, 2D gel of dried dimensions 13 x 15 cm is returned for each sample along with a pH gradient plot and a method description sheet suitable for reports or publications. The example on the next page is from an *E. coli* sample prepared in our Urea sample buffer (left) and SDS Boiling Buffer (right) run identically on a pH 4-8 IEF gradient and 10% acrylamide slab gel. The great advantage of SDS buffer is ease of sample preparation.

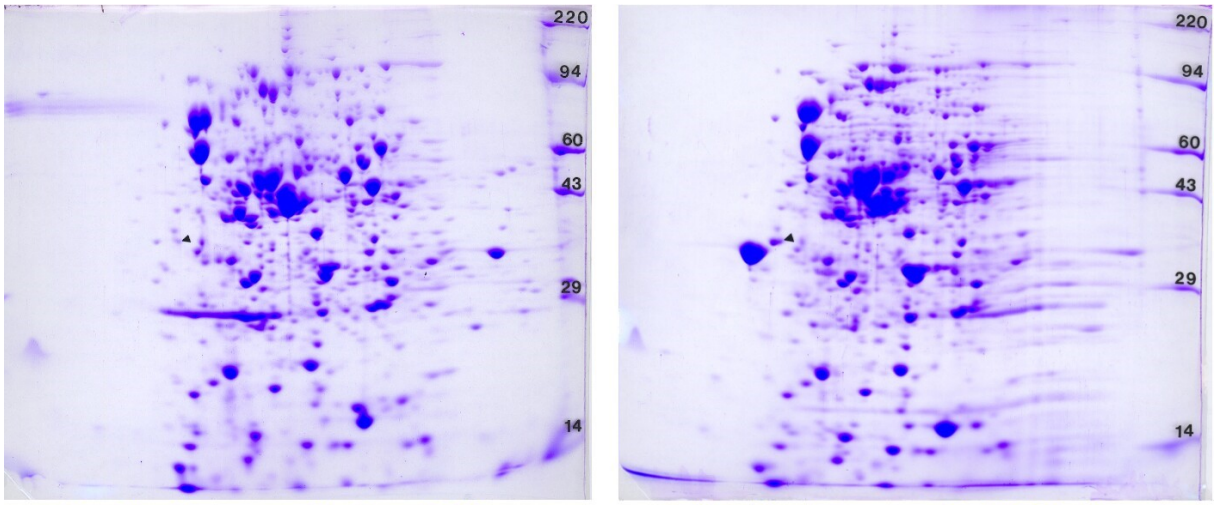
Molecular weight standards (220, 94, 60, 43, 29, and 14 kDa) appear as bands on the right side of the gel labeled electronically on images. One IEF marker, added to each sample as an internal standard, is marked with an arrow.

Our SF IEF tube gel contains either 2% pH 3-10 Isodalt, or 2% pH 4-8 mix, both from Serva.

Transparency drying (**STP-8**) is standard. The duplicate gel rate is used for duplicate gels with different stains and for blots, but not for different gel conditions. More 2D gel examples are on our web page. See **2D-ES-5** for a large format option.

Price:

2D-ES-1 1 sample $293
2D-ES-1A 2-3 samples $270 each
2D-ES-1B 4-8 samples $258 each
2D-ES-1C 9-20 samples $242 each
2D-ES-1D 21+ samples $230 each
2D-duplicate $138 each



E. coli pellet prepared in Urea Buffer E. coli pellet prepared in SDS Buffer

Figure 1. A comparison of 2D gel patterns from the same E. coli sample prepared in Urea (left) and SDS (right) buffer. SDS brings a major protein (~35 kDa) into the pattern but causes some streaking on the basic end (right) of the pH gradient. On the whole 2D SDS PAGE gives better recoveries and linearity of response on 2D gels.

Custom pH Gradients

Available using ampholines of pH 3.5-10, 2.5-5 and 7-9. For basic proteins with pIs greater than 9, non-equilibrium pH gradient electrophoresis is used (NEPHGE, O'Farrell, P. et. al. *Cell 12*: 1133, 1977). Buffers and electrodes are reversed and the pH gradient is created using pH 8-10.5 ampholines. Indicate which custom pH gradient is necessary on the back of the sample ID form.
Price: No extra charge

2D-ES-2 2D SDS PAGE with peptide slab gels

The 16.5% peptide slab gels of Schagger and von Jagow (*Anal. Biochem. 166*: 368, 1987) are useful to resolve low MW proteins (3,000 - 14,000). In general it is better to load low MW proteins heavily (10 µg/spot). Note however, that ampholines may interfere in the MW range 2,000-6,000.
Price: Add $48/gel

2D-ES-3 Semi-native 2D Electrophoresis

For semi-native 2D, denaturing agents urea, SDS, NP-40 and sulfhydryl reagents are omitted from the sample buffer and from the second dimension slab gel. However, 9M urea is a structural component of the IEF tube gel and must be retained during isoelectric focusing. We don't recommend this. Proteins tend to clump under semi-native conditions; some are insoluble and must be centrifuged off. However, in some cases this 2D variation is useful.
Price: Same as 2D-ES-1

2D-ES-4 Non-reducing 2-D SDS PAGE

Same as **2D-ES-1** except sulfhydryl reagents (beta-mercaptoethanol and dithiothreitol) are omitted from sample buffer, internal standards, and all 2D solutions.
Price: Same as 2D-ES-1

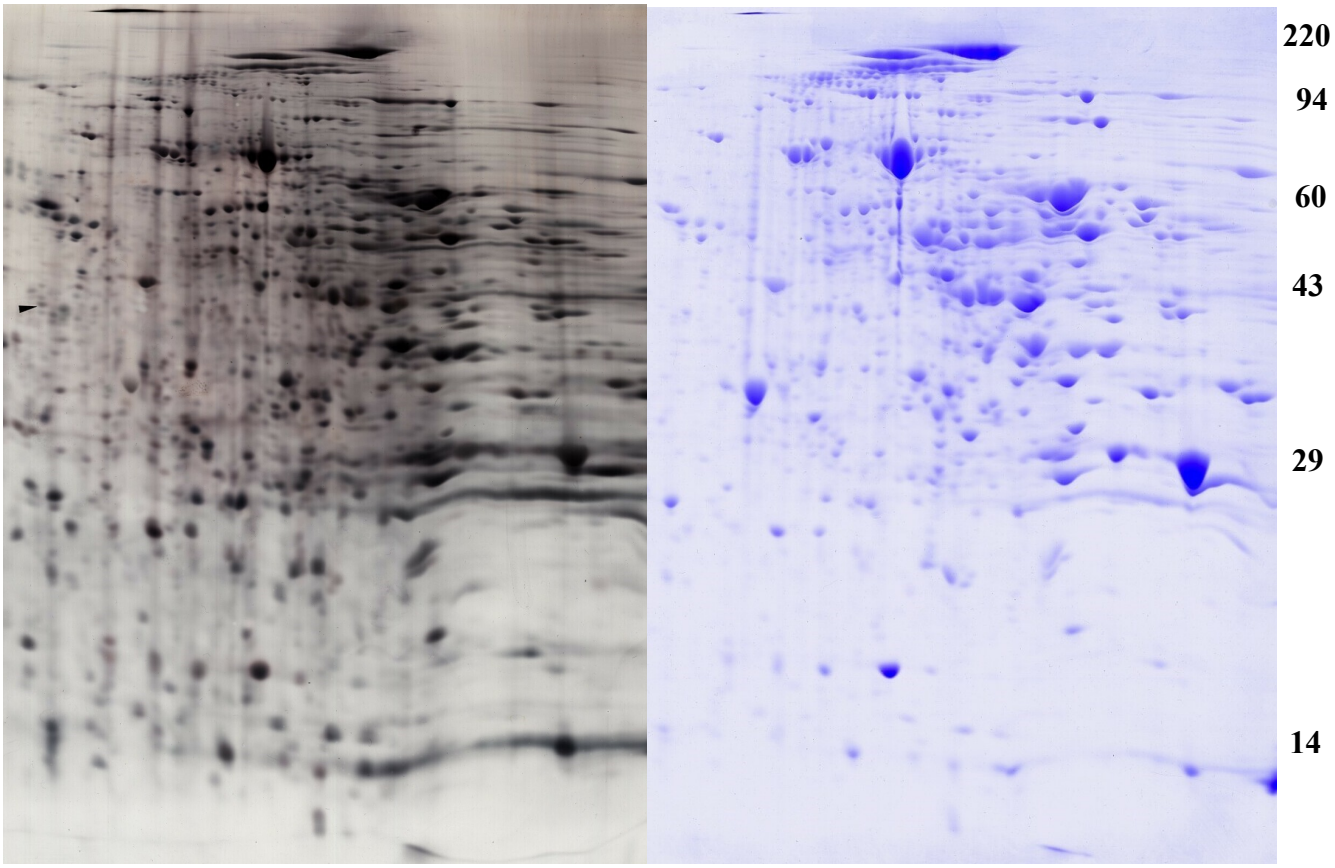


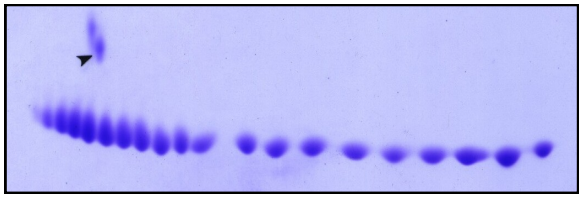
Figure 2. Silver versus Coomassie staining for large format 2D gels run with Isodalt 3-10 ampholines. A side by side comparison of 2D gels loaded with the same rat liver cytosol and stained with silver (left, 100 µg sample load) and Coomassie blue (right, 600 µg sample load). The silver stain is at least 10x more sensitive than Coomassie blue stain even though less is loaded. See page 14 for more information.

2D-ES-5 Large Format 2D SDS PAGE

The larger size gives better resolution for proteomics applications; heavier loads may be applied to obtain more material for mass spectrometry. One Coomassie blue-stained, dried 2D gel of dimensions 20 x20 cm is returned for each sample along with a pH gradient plot and a method description sheet. Our standard IEF tube gel contains 2% pH 3-10 Isodalt from Serva or 2% 4-8 ampholine mix; Custom pH gradients are available. Molecular weight standards (220, 94, 60, 43, 29, and 14 kDa) appear as bands on the right side of the gel. One IEF marker, added to each sample as an internal standard, is marked with an arrow. Transparency drying (**STP-8**) is standard. Typical protein loads are 100 (silver staining) or 600 µg (Coomassie blue) in 25-150 µl.

LF Gel Price:

2D-ES-5	1 sample $430
2D-ES-5A	2-3 samples $387 each
2D-ES-5B	4-12 samples $366 each
2D-ES-5C	13-200 samples $350 each
LF-Dup	LF 2D duplicate gels $178 each



CA Spot #	pI +/- SE	CA Spot #	pI +/- SE
1	7.30±0.05	11	5.70±0.03
2	7.11±0.03	12	5.63±0.03
3	6.97±0.02	13	5.54±0.02
4	6.75±0.02	14	5.46±0.02
5	6.61±0.01	15	5.37±0.02
6	6.45±0.04	16	5.27±0.02
7	6.27±0.04	17	5.20±0.02
8	6.11±0.05	18	5.14±0.02
9	6.01±0.04	19	5.08±0.02
10	5.86±0.04	20	4.99±0.03

2D-ES-7 Carbamylated Carbonic Anhydrase pI Marker (MW 29,000)
This protein was purchased from Sigma-Aldrich, carbamylated at Kendrick Labs by heating in urea, and the pIs of individual isoform measured. It's often quite useful as an internal pI standard for 2D gels. For example, it facilitates matching protein charge shifts on Western blotted immunoprecipitated proteins, when nothing else is on the 2D gel. The carb-CA shows up on the image of each Coomassie-stained PVDF blot that is superimposable on the ECL films. Note that these pIs are not absolute values but are for conditions of 9 M urea and 22° C. The arrow marks an internal standard protein, tropomyosin, MW 33,000, pI 5.3.
Price: Add $34 per gel

Comparisons, Gel Scanning

2D-ES-9-SF/LF Manual Comparisons of 2D Patterns for Differences
We find that HBC (Human Brain Comparison) is a reliable method for finding changes in 2D patterns. In this method two experienced analysts independently compare stained gel pairs on a light box for differences. Results are presented as color coded spot outlines drawn on transparent overlays covering the gels. *Free duplicate gels are run to confirm differences*
Price:
2D-ES-9-SF Standard Format $296 per pair compared
2D-ES-9-LF Large Format $345 per pair compared

2D-ES-10-SF/LF Computerized Comparisons of 2D Gel Patterns for Differences
This analysis includes laser scanning and computerized analysis with SpotMap software from TotalLab, ltd. Requires prior 2D electrophoresis. *Free duplicate gels are run and analyzed to confirm differences for computerized comparisons.* Generally 400-800 spots are quantified per SF gel; 800-2,000 spots are quantified per LF gel. Spot density values are expressed as spot percentages (individual spot density as a percentage of total density in all spots analyzed) to normalize for differences in sample loading or staining. Values for corresponding spots from duplicate gels are averaged. Results are presented in a 2D Comparison Report including a summary table showing spot number, pI, MW, ratios (fold difference) and p values as a second measure of difference. The final report also includes figures of images showing numbering, montage images (picture next page) for every differing protein spot, methods and pH gradient plot. A CD containing data and image files is included in the package. Note that our reports focus on protein differences between samples. Picking protein spots later for identification by mass spectrometry is straightforward. Just send a list of spot numbers and we'll take it from there.
Price:
2D-ES-10-SF Standard Format $775 per comparison
2D-ES-10-LF Large Format $835 per comparison
Lic-1 Image licensing fee Add $125/image

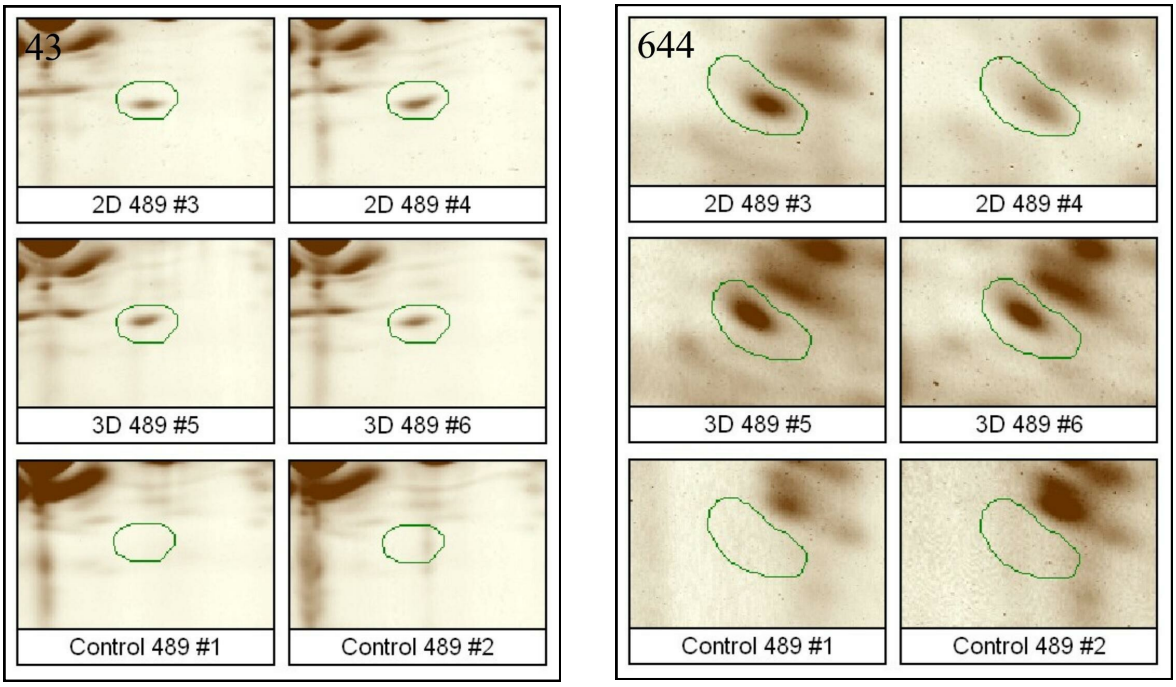


Figure 3. Example of montage images obtained for two protein spots from a 2D gel comparison using SameSpots software. Three samples (2D, 3D and Control) were run in duplicate on silver stained gels. The protein spot number in the upper left corner matches back to the Difference Table 1 in the report where integrated densities and ratios are provided.

2D-ES-12 Quantification of Proteins resolved by 2D Electrophoresis For clients knowing which proteins need to be quantified. Scanning (2D-ES-13) is included. Data is reported in integrated spot density units, or as a percentage of total image density. *Note: Spots are not matched from gel to gel.*

Price:

2D-ES-12 1-12 spots $157
2D-ES-12A 13-50 spots $270
2D-ES-12B 51-200 spots $323
Lic-1 Image licensing fee Add $125/image

2D-ES-13 Laser Densitometry

Silver-stained gels and x-ray films are digitized with our laser densitometer from Molecular Dynamics, linear over 0-3.0 optical density units as verified by calibrated filters. Scanning resolution is 100 microns per pixel; the final black & white tif images are 3.3 Mbytes for standard format and 10 Mbytes for large format.

Price: $44/scan (but included with computer comparisons)

2D-ES-13A Fluorescent Densitometry with a validated Bio-Rad ChemiDoc XRS+ CCD Imager using Bio-Rad Image Lab software.

Price: $73/scan

2D-ES-13B Quantitative densitometry with a GE Healthcare Image Scanner III verified to be linear from 0-2.3 OD using Melles Griot NIST-calibrated filter. Images are scanned at 84 micron resolution.

Price: $44 scan (but included with comparisons)

2D-ES-14 Electronic Photos and Files

The 10 MB black & white files obtained from quantitative densitometry are too big to email and sometimes don't look right by eye. Our GE Healthcare desktop scanner gives excellent color images in tif format that are useful for publication and emailing after conversion to jpg. The price includes image files provided with the dried gels, and jpg images emailed to provide previews of 2D patterns. All gel images are archived in-house.

Price: $25 per scan

HCP Antibody or Drug Substance Analysis using 2D SDS PAGE

Recombinant therapeutic proteins are drugs produced by bioengineered bacteria or cultured cells, the host cells. Over 100 recombinant proteins have been approved by the FDA and many more are being tested. Typically an ELISA assay is used to quantify host cell protein (HCP) contamination of the product. The tests described on this page are used to characterize anti-HCP antibodies chosen for the ELISA. Our tests are also used to verify HCP removal from semi-purified or purified Drug Substance (DS).

In these tests, the number of spots on 2D ECL films from an optimized western blot are compared to the number on silver-stained gel patterns from the same sample. Light and dark films are used to create a detailed report.

For ELISA antibodies, detection of a high percentage of the silver-stained proteins is desirable.

For DS preparations, evidence that the DS contains few or no detectable HCP proteins as determined by an orthogonal method is desirable.

For more information see our 2020 white paper: ELISA CHO HCP Antibodies—Coverage Considerations

The PowerPoint Silver-filmAlignment shows how the silver-stain image is matched to that of the western blot ECL film.

ELISA Antibody Reactivity with Complex Harvest Supernatants, or with Null Cell or DS-containing Whole Cell Lysates

These tests are time-consuming because of 2D pattern complexity (often > 1000 spots) for both silver and western blot images. Any protein spots unique to the western blot images are shown as blue dots on the silver master pattern in the report.

Each 2DE package includes Western blot with light and dark exposures, silver-stained 2D gels in duplicate, electronic photos, and computer comparison with complete report.

Price:
HCP-WCL-SF Standard Format $4025
HCP-WCL-SFA Addtl abs, same SF silvers $3275
HCP-WCL-LF Large Format $4475
HCP-WCL-LFA Additional antibodies, same LF silvers $3630

Semi-Purified or Final Drug Substance Analysis Packages

These packages, similar to those for the ELISA antibody analyses, are useful for confirming product purity by an orthogonal method to mass spectrometry and ELISA testing. Note that DS breakdown products are detected by comparison with an additional western blot (HCP-PSR-LFR below) if necessary.

Purified or Semi-purified Drug Substance Analysis Packages Each 2DE package includes Western blot with light and dark exposures, silver-stained 2D gels in duplicate, electronic photos, and computer comparisons with complete report.

Price:
HCP-DS-SF Standard Format $2960
HCP-DS-SFA Addtl antibodies, same SF silvers $2370
HCP-DS-LF Large Format $3270
HCP-DS-LFA Addtl antibodies, same LF silvers $2665

Product Spot Removal

HCP-PSR-LFR Removal of product spots from the HCP-LF report. Includes an additional western blot with anti-product antibody. **Price:** Add $1260

HCP-PSR-LFRA Removal of product spots from report for additional HCP antibody using product western blot from HCP-LFR. **Price:** Add $625 each

Run Documentation

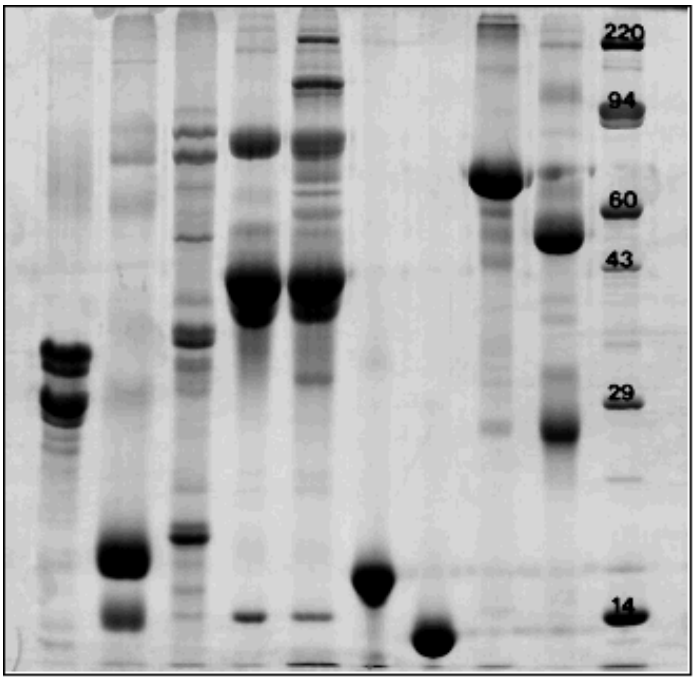
GTRK-1 Run documentation including IEF, slab gel and WB checksheets for HCP-SF/HCP-LF antibody analyses along with a*rchival of reports and data files on the password protected cloud storage site Egnyte.*
Price: Add 30%

HCP Antibody Screening using 1D Electrophoresis (SDS PAGE)

Anti-HCP antibodies are polyclonal, usually from rabbits or goats, because the antigenic protein mixture is complex. 1D-HCP is a good way to check the time course of HCP antibody production and to compare reactions of different animals.

1D-HCP-1 1D package includes HCP sample preparation, standard format SDS PAGE (13 x 15 cm gels, 15 wells) western blot with multiple exposures, Coomassie blue stained gel, electronic images, and a printed report showing images. Visual interpretation, usually straightforward, is performed by the client unless otherwise requested. **Price:** $770
1D-HCP1A Additional antibodies tested against the same antigen. **Price:** $532 each

1D (Slab Gel) Electrophoresis



1D-ES-1 SDS Slab Gel Electrophoresis Option of 10, 12, 15, or 20 wells per comb. Includes weighing and dissolving samples; 2 lanes/sample. Example photo above shows food proteins. *Lanes 1-9, left to right: Lane 1, casein; 2, whey; 3, soy; 4, egg white; 5, whole egg; 6, beta-lactoglobulin; 7, alpha-lactalbumin; 8, bovine serum albumin; 9, IgG; 10, molecular weight markers.*
Price:
1D-ES-1 $193 for first sample
1D-ES-1A $94 each for additional sample

1D-ES-2 Quantification of Proteins Resolved by 1D Includes scanning and quantitative analysis. Results are reported in a table showing % total stain density for each band in a gel lane (3 lanes/sample) plus printouts of lane density profiles and images. Note that the molar ratio of stain/protein may vary between proteins. This is a good way to compare similar samples but not good for determining % protein by weight.
Price: Add $135 per sample

1D-ES-3B Molecular Weight Distribution Plot Requires 1D-ES-1. Includes scanning & analysis.
Price: Add $94 per sample

1D-ES-4 Double Thickness (1.5mm) Slab Gels To maximize material loaded for transblotting and subsequent sequencing.
Price: Add $61 per gel

1D-ES-5 Peptide Slab Gel for resolution of low molecular weight (2000-12,000).
Price: Add $48 per gel

1D-BN-PAGE Blue Native Polyacrylamide Gel Electrophoresis is a charge shift method for isolation and characterization of large multi-protein complexes (MPCs) in their native state. Novex Blue native minigels are used.
Price:
1D-BN-PAGE $193 first sample
1D-BN-PAGE-A $94 each additional

1D-IEF Isoelectric focusing is useful for examining protein charge differences. Novex IEF minigels are used.
Price:
1D-IEF $193 first sample
1D-IEF-A $94 each additional

1D Electrophoresis Quantification Packages These packages are used to quantify proteins relative to a standard curve for determining percent of a known protein by weight in a sample. An example is the determination of percent casein and whey in dairy products. Each sample is weighed along with standards and dissolved by boiling in SDS buffer to a known concentration. A set of standards (4 concentrations in duplicate) is run with 2 concentrations of sample (in duplicate) on two gels. The gels are scanned, standard curves are generated, and the concentration of the unknown in the sample is determined from the standard curve. A report giving the concentration (for example casein percentage by weight), along with a description of the method, is mailed out. Unless otherwise requested, dried gels are filed.
Prices: See Table on page 11.

Percent Casein and Whey Protein by Weight

Each sample is weighed and dissolved by boiling in SDS buffer to a known concentration. A set of standards (4 concentrations in duplicate using casein and whey standards) is run with 2 concentrations of sample (in duplicate) on two electrophoresis gels. The gels are scanned with a calibrated densitometer and the images analyzed using TotalLab software (TotalLab Ltd) on a Windows 10 computer. Standard curves are generated and the concentration of casein and whey in the sample determined from the standard curves. A report giving the concentrations along with a description of the method is emailed and a printed copy mailed. Unless otherwise requested, dried gels are filed.

Percent Lactoferrin by Weight

Includes quantitative measurement of lactoferrin relative to a standard curve. The method and report are as described for % Casein and Whey by weight.

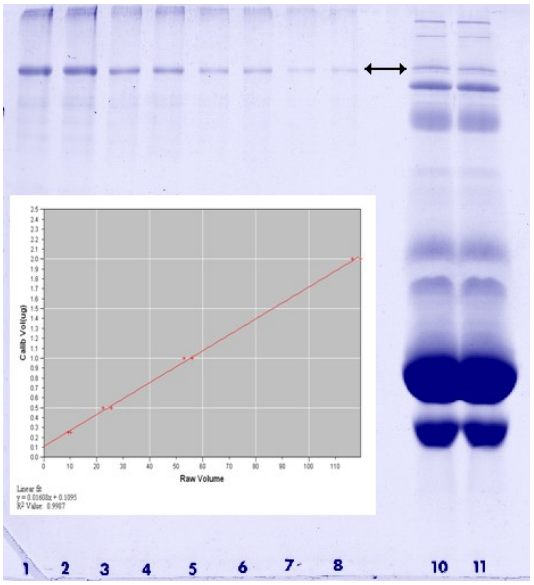


Figure 4. Percent Lactoferrin by Weight in Whey Sample. The double arrow marks the lactoferrin band in the standard curve (8 lanes on left) and in the unknown whey sample (two lanes on right). The plot shows integrated density of lactoferrin band (duplicate values averaged) versus amount loaded. R^2 value for the curve is 0.9987.

Relative Amount of Whey Components

Includes measurement of the Coomassie blue stain contained in the alpha-lactalbumin, beta-lactoglobulin, bovine serum albumin, lactoferrin, and immunoglobulin bands. The samples are analyzed by SDS slab gel electrophoresis in triplicate, the stained gels digitized with a laser densitometer, and computerized quantitative analysis of stain density of individual protein bands carried out. Results are presented in a table showing % total stain density (average of triplicate measurements) for each protein band. Note: This method is best used for comparing similar samples since the stain/protein ratio may vary from protein to protein. It gives relative values rather that absolute percent protein by weight.

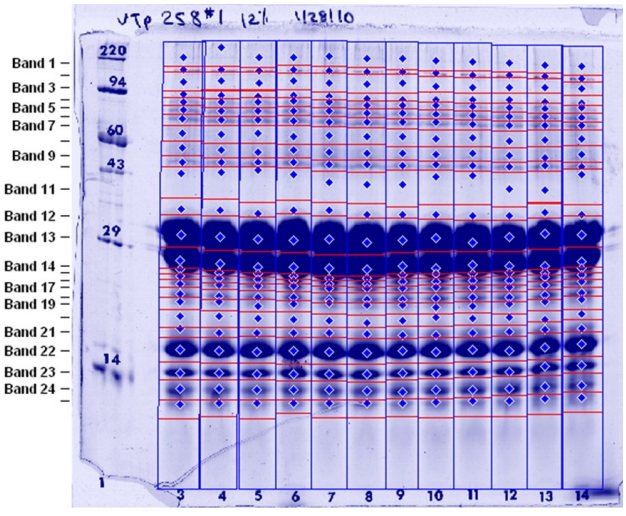


Figure 5. Band outlining for relative amount of whey protein by weight. The bands are identified in the report if possible.

Electrophoresis Assays for Food Proteins				
Item #	**Description**	**Qualitative or Quantitative**	**Lower Limit of Sensitivity**	**Price**
1D-PKG-1	Percent any protein by weight, client must supply purified protein for standard curve	Quantitative	depends	1-2 $477 ea 3-9 $415 ea >10 $383 ea
1D-CW 1D-CW-A 1D-CW-B	Percent Casein/Whey by weight from standard curves	Quantitative	<1.0%	1-2 $286 ea 3-9 $223 ea >10 $205 ea
1D-CPR 1D-CPR-A	Relative amount of individual casein components. (stain density of α, β, and K casein)	Quantitative but results expressed in relative terms.	<0.5%	First $287 Addtl $188
1D-WPR 1D-WPR-A	Relative amount of whey proteins (stain density of α-lactalbumin, β-lactoglobulin, lactoferrin, BSA, & IgG)	Quantitative but results expressed in relative terms.	<0.01%	First $287 Addtl $188
1D-MPR 1D-MPR-A	Relative amounts of milk proteins (both casein and whey)	Quantitative but results expressed in relative terms.	depends	First $287 Addtl $188
1D-TC 1D-TC-A	Trace Casein Detection	Qualitative	< 1.0%	First $456 Addtl $208
1D-SP 1D-SP-A	Trace casein detection using electrophoresis + Western blotting	Qualitative	< 0.005	First $925 Addtl $355
1D-TCW 1D-TCW-A	Trace milk detection (casein and whey) using electrophoresis + Western blotting	Qualitative	<0.005	First $1025 Addtl $615
1D-ES-3 1D-ES-3-A	MW distribution plot for hydrolyzed samples	N/A	N/A	First $287 Addtl $188
1D-EW 1D-EW-A	Egg white or whole egg detection	Qualitative	< 1.0%	First $287 Addtl $188
1D-LF 1D-LF-A 1D-LF-B	Lactoferrin by electrophoretic analysis with standard curves. This assay is useful for powders.	Quantitative	<0.1%	1-2 $347 ea 3-9 $296 ea >10 $245 ea

Table 1. Electrophoresis Assays Available for Food Proteins. The sensitivities for these assays vary with interference and may be either greater or lower depending on the sample. Please call to get estimates for your particular sample type. Qualitative results are expressed in yes/no terms i.e. the protein being tested is either present or absent from the sample within the limits of sensitivity of the assay.

Staining/Drying Procedures

STP-1 Coomassie Blue Staining

The classic Coomassie brilliant blue R250 staining technique (O'Farrell, P. *J. Biol. Chem. 250*: 4007, 1975) is very sensitive. The method involves an alcohol/acetic acid fix, an acetic acid rehydration, and an acetic acid destain. Generally, 1 µg of purified protein gives a dark spot on a 2D gel (see our internal standard); 0.1 µg is clearly visible. Although not as sensitive as silver staining, Coomassie blue is quantitative and reproducible for proteins resolved by both 1D and 2D electrophoresis (Kendrick et. al. Adv Exp Med Biol 2019 Vol 1140 pp563-574). This stain is compatible with mass spectrometry.

Price: No charge (Default stain)

STP-2 Silver Staining

Our silver stain is based on the ammoniacal silver/formaldehyde method of Oakley et. al. (Anal. Biochem.105: 361, 1980.) This method involves preliminary glutaraldehyde treatment of the slab gel to fix proteins by cross linking. The pretreatment also adds glutaraldehyde side chains to the proteins, increasing sensitivity since these groups are sites for silver deposition (Dion A, and Pomenti A, *Anal. Biochem. 129:* 390, 1983). We find this method to be about 10 times more sensitive than Coomassie blue staining, depending on the protein, even though less protein is loaded. Generally, 50 ng of purified protein gives a highly visible spot on a 2D gel (see our internal standard). However, some proteins that are detectable with Coomassie don't stain at all with silver. This is a semi-quantifiable stain in that different proteins saturate at different levels, e.g. some at 100 ng, some at 300 ng. This silver stain is not compatible with mass spectroscopy because of the glutaraldehyde.

Price:

STP-2 Standard Format Add $61 per gel
STP-2LF Large Format Add $73 per gel

STP-3 Special Silver Staining for Mass Spectrometry

Special silver staining (O'Connell and Stults, *Electrophoresis 18*: 349-359, 1997) omits the glutaraldehyde step and is compatible with mass spectroscopy. It is not suitable for computer analysis because many protein stain negatively. However, the pattern can be matched to our regular stain without difficulty. Note that silver deposition interferes with the enzymatic digestion required for mass spectrometry for most mammalian proteins. If possible use the less sensitive but safer Coomassie stain.

Price:

STP-3 Standard Format Add $61 per gel
STP-3LF Large Format Add $73 per gel

STP-4 Sypro Ruby Fluorescent Stain

This Life Technologies stain fluoresces linearly with protein over a range of 100-1000 ng and is compatible with mass spectrometry.

See 2D-ES-13A Fluorescent densitometry (page 8) for imaging.

Price:

STP-4 Standard Format Add $115 per gel
STP-4LF Large Format Add $152 each

STP-5 Cy dye staining for DIGE analysis

Inquire for quote

STP-8 Transparency Drying:

All stained gels are air dried between cellophane sheets unless otherwise requested. Although this treatment adds a day to turnaround, the advantages of easier storage, little or no curling, good color retention, overlay capability, scanning capability, and overhead projection capability offset the time delay.

Price: No extra charge

STP-9 Paper Drying

This is an optional drying method for stained gels.

Price: $16 per gel

STP-10 Return of Wet Gels

Wet gels are placed between sheets of thick wet filter paper and the sandwich placed in a ziploc bag. The bag is supported by pressboard and bubble wrap for express mail.

Price: Add $34 to shipping

Blotting Procedures

BP-1 Transblotting onto PVDF for your lab

Transblotting is carried out overnight according to standard procedures using a CAPS/methanol buffer system without SDS. The dried stained (BP-2) or unstained blot is returned to the client. Staining facilitates matching to a duplicate Coomassie gel for mass spectrometry. Original gels are stained to make sure that they are blank and then discarded, unless otherwise requested.

Price:
BP-1 Standard Format gel $116 per blot
BP-1LF Large Format gel $133 per blot

BP-2 PVDF Coomassie Staining

The 2D gel pattern obtained from Coomassie blue staining of a PVDF membrane is faded but the scanned image (can be contrasted with Adobe photoshop) is exactly superimposable with the ECL film from Western blotting and allows for the matching of a few spots on the film to a busy 2D gel pattern on a duplicate Coomassie gel run for mass spectrometry. Since Western blotting can be 100 times more sensitive than Coomassie, exact matching is very important. Coomassie staining of the blot does *not* interfere with subsequent Western blotting.

BP-2 Price: Add $22/blot

2D-ES-14 Electronic photos (p8)

Price: Add $25/blot

BP-3 Transblotting onto Nitrocellulose

Transblotting is carried out overnight according to standard gel procedures using a tris/glycine/methanol buffer system containing 0.1% SDS, with nitrocellulose of 0.45 micron pore size. Dried unstained blots are returned to the client and original gels are discarded unless additional treatments are requested. We will change the blotting procedure to your specifications if necessary. Nitrocellulose cannot be Coomassie blue-stained.

Price:
BP-3 Standard Format $116 per blot
BP-3LF Large Format $133 per blot

BP-4 Pre-Stained Molecular Weight Markers for Blots

These markers, Precision Plus Protein Standards from BioRad, are added during slab gel electrophoresis so that colored bands are visible down the edge on both unstained gels and subsequent transblots at molecular weights of 250, 150, 100, 75, 50, 37, 25, 20, 15 and10 kDa.

BP-4 Price: $27 per gel

BP-6 Western (Immuno-) Blotting

This method for immuno-detection of proteins is carried out on PVDF transblots from 1D or 2D gels according to standardized operatoring procedures. **Requires prior electrophoresis** (2D-ES-1 or 1D-ES-1) but PVDF transblotting (BP-1) is included in the fee. Generally the client supplies the antibody. The sensitive ECL method of detection is used.

Price:

BP-6SF	Standard Format	Add $485/sample
BP-6SF-Dup		Add $333
BP-6LF	Large Format	Add $605/sample
BP-6LF-Dup		Add $460

Specialty western blotting of post-translational modifications:

- **Phosphotyrosine (BP-7)**
- **Phosphoserine & phosphothreonine (BP-8 & 9)**
- **Acetyl-lysine (BP-10)**

BP-7 Phosphotyrosine western blotting

Phosphotyrosine western blotting with the PY20 antibody has proven to be remarkably sensitive, specific and reproducible (PLoS One 2020 Vol. 15 Issue 6 Pages e0234645). **Package includes** 2DE, PVDF transblotting, blot Coomassie staining, immuno-staining with PY20 and electronic photos.

Package Price:

BP-7SF	Standard Format gels	$575/sample
BP-7SF-Dup	SF duplicate package	$394/sample
BP-7LF	Large Format gels	$652/sample
BP-7LF Dup	LF duplicate package	$485/sample

BP-8 Phosphoserine/phosphothreonine 2D western blotting includes ECL Ultra and duplicate blots but not antibodies

Our optimized method for pSer/pThr 2D western blotting uses ECL Ultra from Lumigen in combination with Qiagen Q5 (pSer) and Q7 (pThr) antibodies. Both antibodies are said to detect phosphorylated residues irrespective of surrounding amino acids. Even so, control sample should be run for purposes of comparison. The high sensitivity of ECL Ultra adds variability so duplicate western blots are recommended. Antibodies are purchased fresh at cost for each client. Inquire to discuss your project and for a quote.

BP-9 Phosphoserine 2D western blotting performed as described for BP-8

Inquire to discuss your project and for a quote.

BP-10 Acetyl-lysine Western Blotting Package

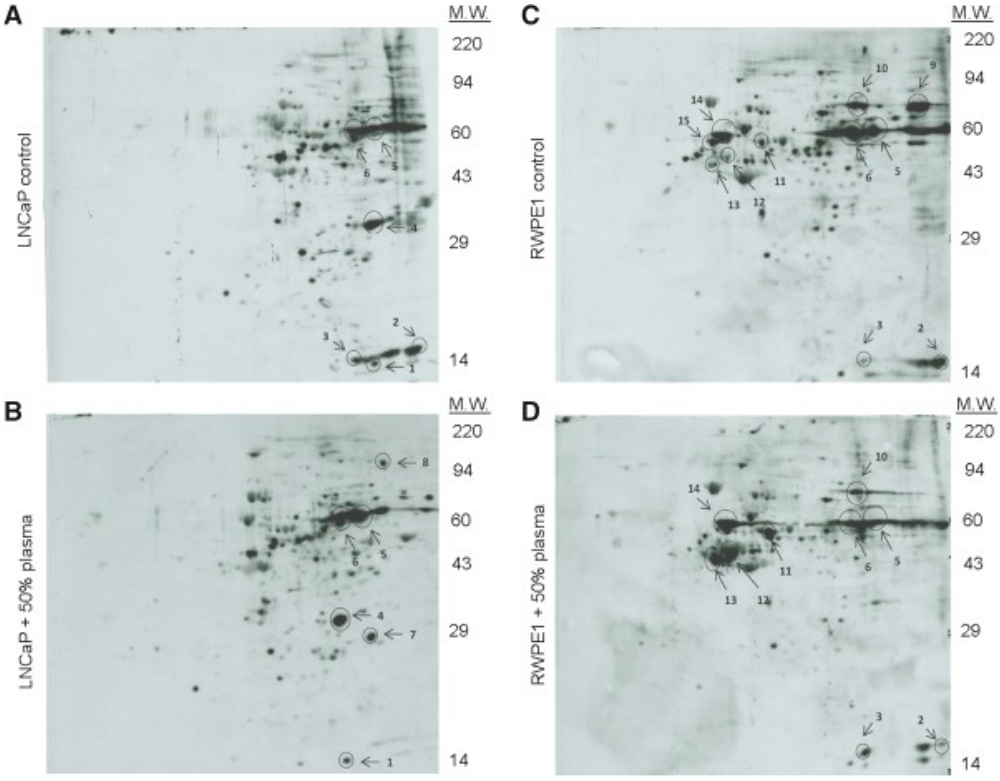
Lysine acetylation is an important post translational modification occurring on many proteins. 2DE with Western blotting allows you to identify acetylated proteins in a complex mixture using polyclonal antibody 9441 from Cell Signaling. **Package includes** 2DE, transblotting to PVDF, blot Coomassie staining, subsequent immunostaining with anti-acetyl-lysine antibody, and electronic photos of stained blot and ECL film.

Price:

BP-10SF Standard Format $700/sample
BP-10SF-Dup Duplicate SF Package $560/sample
BP-10-LF Large Format $895/sample
BP-10LF-Dup Duplicate LF package $700/sample

For examples of acetyl-lysine WB see:

1. Kim, S.Y., Q. Zhang, R. Brunmeir, W. Han, and F. Xu, SIRT1 Interacts with and Deacetylates ATP6V1B2 in Mature Adipocytes. PLoS ONE, 2015. 10(7): p. e0133448.
2. Mitra, R., O.B. Goodman, and T.T. Le, Enhanced detection of metastatic prostate cancer cells in human plasma with lipid bodies staining. BMC Cancer, 2014. 14: p. 91. (**below**)
3. Shepard, B.D., D.J. Tuma, and P.L. Tuma, Chronic ethanol consumption induces global hepatic protein hyperacetylation. Alcohol Clin Exp Res, 2010. 34(2): p. 280-91.



Taken from: Mitra, R., Goodman, O., and Le, T. Enhanced detection of metastatic prostate cancer cells in human plasma with lipid bodies staining. BMC Cancer, 2014. 14: p. 91.

Their Figure 2. 2D Western blots of lysine acetylation profiles of (A) untreated LNCaP cells, (B) LNCaP cells incubated with human plasma, (C) untreated RWPE1 cells, and (D) RWPE1 cells incubated with human plasma.

Example of BP-10: Acetyl-lysine western blotting

Sample Preparation Procedures

SP-1 Protein Determination followed by buffer addition. The total amount of protein in each sample is determined by the Pierce BCA assay. *If you require protein determinations, do not dissolve samples in buffers containing interfering reducing agents like dithiothreitol or beta-mercaptoethanol (BME).* We will add BME later.
Price:
SP-1 BCA assay $60/sample
SP-1B Bradford Assay $60/sample

SP-2 Combining multiple sample tubes, for example from an immunoprecipitation. This involves transferring each tube's contents, adding a rinse, vortexing, and transferring the rinse.
Price: 1-4 tubes, no charge
5-10 tubes, Add $73/sample
11-20 tubes, Add $145/sample

SP-3 Ethanol Precipitation of Proteins followed by buffer addition. See our web link: www.kendricklabs.com/EtOHppt_2008.pdf For more information.
Price: $39/sample.

SP-4 Homogenization includes homogenization with osmotic lysis buffer containing protease inhibitors followed by incubation on ice with DNase and RNase to break down interfering polynucleotides. The composition of the 10X Nuclease Stock Solution is given in our Sample Preparation Guide. If you require nuclease treatment send the samples in a buffer containing less than 0.3% SDS. Higher SDS concentrations inactivate nucleases. **Price:** $55/sample

SP-5 Microdialysis followed by lyophilization & buffer addition. High salt concentration (> 150 mM) interferes with IEF; the less salt the better. If your samples contain high salt or buffer (phosphates, NaCl, etc.), this dialysis and concentration step is highly recommended.
Price: $44/sample

SP-6 Lyophilization followed by buffer addition. **Price:** $39/sample

SP-7 TCA Precipitation followed by buffer addition. **Price:** $39/sample

SP-8 TCA/Acetone Precipitation followed by buffer addition. **Price:** $39/sample

SP-9 Custom Sample Preparation is charged by the hour. **Price:** $242/hr

SP-10 Albumin/IgG Removal From Serum, Plasma & CSF of Human, Rat & Mouse
This service is performed using the Millipore Sigma ProteoExtract Albumin/IgG Removal kit Cat. # 122642. Depletion of albumin and IgG from 20-60 µl using the disposable gravity-flow affinity columns removes up to 75% of total serum protein so that 3-4 times more enriched sample can be loaded.
Price: $92/sample

SP-11 BioRad Ready Prep for 2D samples:
Price: $114/sample

Regarding Sample Preparation for 2D SDS PAGE

- **SDS Buffer is fine.** The carrier ampholine tube gel system used for 2D SDS PAGE is compatible with 2.5% SDS. See our book chapter Kendrick et. al. Adv Exp Med Biol 2019 Vol 1140 pp563-574) for more info.
- **1D Samples work well.** Any sample that you've previously prepared for SDS PAGE gels can usually be run on our 2D gels as long as the protein concentration is known and salt is < 200 mM. To make sure, send a 1D gel image to Jon Johansen, Lab Manager, (jon@kendricklabs.com) with lanes IDs.
- **IP Bead Prep is free.** We will take immuno-precipitated proteins off your IP beads without charge prior to running 2D gels. Just take the supernatant off and send the beads on dry ice with a sample ID form.
- **Don't hesitate to call for advice** if you'd like to prepare samples in your lab. Buffer recipes are posted on our web site or order our Mailing Kit (MK-1, page 4). If there's a problem with sample preparation, we'll do free reruns to optimize the pattern.

Protein Identification by Mass Spectrometry

We have considerable experience in sending protein spots cut from 2D gels to university and commercial core facilities for identification by mass spectrometry (MS.) Turnaround time for protein identification by LC/MS/MS is usually about two weeks. We highly recommend the Clarkson University Protein Core. Please contact Dr. Costel Darie, the facility director, for more information. (tel: 315-268-7763, email: cdarie@clarkson.edu)

2D-ES-19 Spot Cutout for MS

We do this manually and are obsessively careful. The cutout spots are placed in Eppendorf tubes for express mail to the core facility of your choice; default is the Clarkson University Proteomics Lab in Potsdam, NY. The cost includes a cover letter to the core listing spots, a copy of the letter for you along with an image showing spot locations. We make sure each spot has a unique identifier.

Price: $34 per spot

2D-ES-18 Mass Spectrometry using nano LC-MS/MS

This powerful method is used to identify proteins cut from Coomassie blue or special silver-stained gels. The cut protein spots are digested with trypsin. Digestion mixture peptides are separated on an LC Packings nano-lc on which the detector outlet is connected directly to the nanospray source of a Micromass Q-Tof mass spectrometer. Peptides are eluted at a flow rate of 200 nl/min and are scanned as they enter the source. When a peptide is detected, the Q-Tof is programmed to switch to MS/MS mode, which means that the eluting peptide is fragmented by means of an applied collision energy and the resulting ions scanned for several seconds. When the programmed time for MS/MS is done, the Q-Tof switches back to MS mode and resumes scanning the eluting peptides.

Because peptides fragment at the peptide bond in a predictable way, the fragmentation pattern can be used to deduce sequence information. The ions resulting from fragmentation along with the mass of the intact peptide can be used to search a database and identify the protein.

Price:

2D-ES-18	1-10 $296 each
2D-ES-18A	11 or more $260 each

2D-ES-18-PTM Identification of post-translational modifications (PTM) using bioinformatics software

Once proteins have been identified by LC-MS/MS, analysis of PTMs may be performed using bioinformatics software. PTMs that can be targeted include methylation, acetylation, phosphorylation, sulfonylation, glycosylation, ubiquitination, sumoylation, and disulfide bridges.

In most experiments, the protein investigated has two states for a PTM site: unmodified and modified. Data analysis is performed with a specially constructed database, which will look for PTMs within the protein using unbiased searches (100% chance for an amino acid to be both modified and unmodified). If the amino acid is modified, the modified peptide will be identified, but not the unmodified one and vice versa. Assuming the peptides fly, PTM's can usually be identified. Other mass spectrometry methods especially designed for PTM analysis such as product ion discovery or neutral loss can also be performed.

Price: $710 per sample

Includes analysis of LS-MS/MS spectra from modified and unmodified samples for up to 3 PTMs. Requires prior 2D-ES-18 for both modified and unmodified samples.

Please call Dr. Darie (315-268-7763) to discuss PTM analysis before sending samples.

2D-ES-20 Consulting fee for custom interpretation of complex spectra by Clarkson Personnel.

Price: $242/hr

Terms and Conditions

A valid purchase order or credit card number must be included on the sample ID form (or called in) before results are returned.

1. FREIGHT - All charges for sample shipment, product shipment, and return of data will be paid for by the customer. Data will be shipped by UPS 2-day express mail at a cost of $50/package or overnight UPS for $65/package unless otherwise specified by customer.

2. PAYMENT - Payment in full shall be made to Kendrick Laboratories, Inc. 1202 Ann St., Madison, WI 53713 (608-258-1565) within 30 days of the invoice date. Payments not received within such 30 day period shall bear interest at the rate of 1.0% per month (12% per year) but not in excess of the maximum amount permitted under applicable law.

3. WARRANTY - Kendrick Laboratories, Inc. products are warranted to conform to the specifications appearing on the product labels, or for dated products, until the date appearing on such label. Kendrick Laboratories, Inc. warranty obligation is limited to replacement of the defective product or the refund of the purchase price, at the option of Kendrick Laboratories, Inc.

The foregoing warranty is the only applicable warranty. All other warranties, expressed or implied, including but not limited to, the implied warranty of merchantability or fitness for any particular purpose are disclaimed.

4. LIMITATION OF LIABILITY - Kendrick Laboratories, Inc. will accept limited liability for defective gels as determined by Kendrick Laboratories, Inc. to be defective. Kendrick Laboratories, Inc. liability shall not include the costs of preparing the new samples or the costs incurred in preparing the original samples. Kendrick Laboratories, Inc. liability shall be limited, in any event, to re-running defective gels at the company's expense, excluding any cost of shipment if fresh samples are required.

It is understood and agreed that Kendrick Laboratories, Inc. liability whether in contract, in tort, under any warranty, in negligence or otherwise shall not exceed the return of the amount of purchase price paid by the purchaser and under no circumstances shall Kendrick Laboratories, Inc. be liable for special, indirect or consequential damages.