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Kendrick Labs Update

www.kendricklabs.com

Volume 2, Issue I

August 2007

NEW! RADical approach to finding protein differences between tissue, serum or organelle sample pairs.

Reciprocal Affinity Depletion (RAD) Method to find proteomic differences between tissues

Normal mouse homogenate

Immunize chickens

Purify chicken antibodies against normal mouse proteins

Make an affinity column

Proteins unique to diseased tissue pass

through in the eluent. High abundance

proteins stay on the column.

Pass diseased mouse homogenate through anti-normal column



Reciprocal Affinity Depletion

Diseased mouse homogenate

Purify chicken antibodies against diseased mouse proteins

Immunize chickens

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Make an affinity column



Pass normal mouse homogenate through anti-diseased column

Proteins unique to normal tissue pass through in the eluent. High abundance proteins stay on the column.

The eluents are greatly enriched in proteins differing between the tissues. Comparison by 2D gel electrophoresis to find differences becomes straightforward as does subsequent identification of differing protein spots by mass spectrometry.

Kendrick Labs Inc is offering **RAD** Technology in collaboration with GeneTel Labs (www.genetel-lab.com). GeneTel personnel will prepare chicken antibodies against your samples, generate reusable antibody columns, and pass the sample(s) over the columns. Kendrick Labs will dialyze the samples, measure protein concentration, run 2D gels, compare the patterns, and arrange for identification of changing proteins by mass spectrometry (MS). Price: \$3600 for RAD, sample preparation, 2D gels/computer analysis, electronic images and complete report for 1 pair of samples. MS is additional.

Dr. David Huang of GeneTel Labs, who developed the RAD method (patent pending), has determined that protein depletion by the affinity columns depends on protein abundance and epitope number. Mammalian proteins in high abundance are selectively removed by the chicken antibodies as well as highly antigenic proteins in low abundance. The effluents from the reciprocal affinity columns contain a complex mixture of proteins enriched for differences. Over for 2D gel images from an example:

David Huang, Ph.D. GeneTel Labs LLC dhuang@genetel-lab.com 877-248-4316





Nancy Kendrick, Ph.D. Kendrick Labs Inc nancy@kendricklabs.com 800-462-3417

Ask to be on our mailing list:

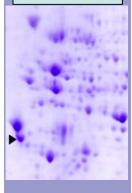
- To be notified of new developments quickly.
- ◆To receive coupons and beta test offers.

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Examples of 2D gels from RAD columns

Message from Jon Johansen, Lab Manager

UNRAVEL YOUR
PROTEOMIC
PROBLEMS
WITH 2D GELS
FOLLOWED BY
MASS
SPECTROMETRY



2D gels from RAD and original samples

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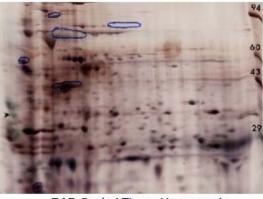
Kendrick Labs Inc 1202 Ann St Madison, WI 53713 800-462-3417 Local: 608-258-1565 Fax: 608-258-1569 2d@kendricklabs.com www.kendricklabs.com

Call or email for a price quote or to discuss your project.

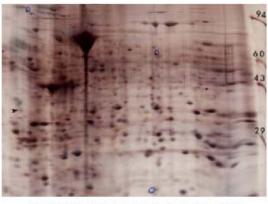


To receive a free 2D gel with your order (of at least 2 gels) note NL:2-1 Coupon on your sample ID form. Includes silver staining but not computer comparisons. Expires 1/1/08.

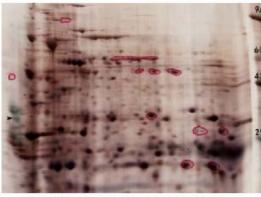
Check out recent references citing Kendrick Labs www.kendricklabs.com/ References-clients.htm



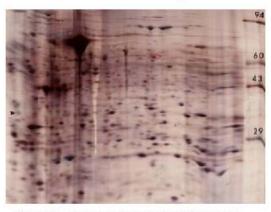
RAD Control Tissue Homogenate



Control Tissue Homogenate before RAD



RAD Diseased Tissue Homogenate



Diseased Tissue Homogenate before RAD

Figure 1. 2D gel patterns from RAD and original samples from mammalian tissue homogenates (with permission). The client has requested anonymity and non-disclosure of the tissue and disease pending publication. Protein spots unique to or enriched in the control sample by eye are outlined in blue while those unique to or enriched in the diseased sample are outlined in red. All differences were confirmed on duplicate 2D gels. Only 3 subtle differences were observed for the original samples, while 14 strong differences were observed for the RAD samples. A computerized comparison of the RAD 2D gels (duplicate gels/sample) is in progress.

Message from our Lab Manager, Jon Johansen:

2D gels are especially useful for studying post-translational modifications because they can detect single charge changes. Evidence of this is shown below. GE Healthcare's carbamylated CPK pI standard was run on our 2D gels with and without SDS. The creatine phosphokinase had been heated with urea buffer so that positively charged lysines were blocked one-by-one by carbamylation. The charge isoforms resolve well on 2D gels. The patterns +/- SDS are identical because SDS is completely stripped from the protein during isoelectric focusing.

5% SDS buffer

9 M urea buffer

Many people use Western blotting to detect new charge isoforms. Western blotting is 10-100 times more sensitive than other staining methods and gives beautiful results. Recently we have been focusing on phosphoprotein Western blotting using the PY20 antibody for P-Tyr and the Qiagen Q5/Q7 antibodies for P-Ser and P-Thr. See the links under "Western blots" on our web page for details.

As always, call or email either me or Nancy to discuss your project or for a quote. Our goal is to bring your project to a successful conclusion so that you'll tell your friends. Once we agree on a project, Klabs will

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hustle for you.