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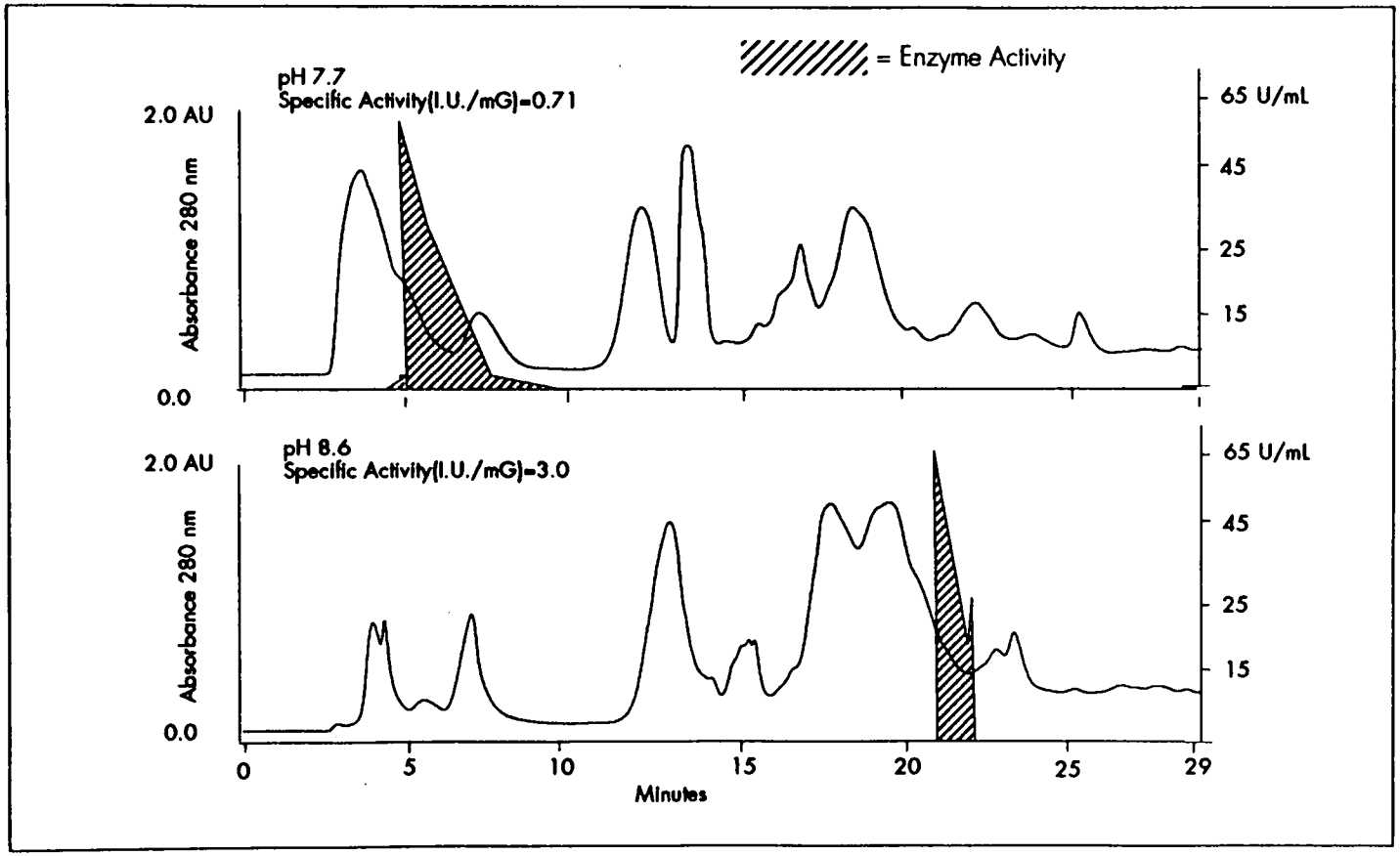
Auto•Blend™ Purification of Mouse Liver Lactate Dehydrogenase



Conditions:

Column: Waters Protein-Pak™ DEAE 8HR (10 x 100mm)

Flow Rate: 1.5ml/min.

Buffer: A: 100mM Tris-HCl
B: 100mM Tris Base
C: 1.0M Sodium chloride
D: MilliQ® Water

pH 7.7

Time	%A	%B	%C	%D	Curve
IN	15	5	0	80	*
20	15	5	25	55	6

pH 8.6

Time	%A	%B	%C	%D	Curve
IN	5	15	0	80	*
20	5	15	25	55	6

Detection: 280nm

Injection: 750µl mouse liver supernatant. (Homogenized in 20mm Tris-Cl, pH 7.5 (3ml/gm), centrifuge at 48,000g for 30 min.@4°)

The use of Waters Auto•Blend Method for optimizing protein purification schemes in combination with Protein-Pak ion exchange columns allows for rapid determination of the pH at which maximum resolution and recovery can be achieved.

Objective:

The objective of this application note is to demonstrate an automated methods development for the selection of the best gradient conditions with Waters 650 Advanced Protein Purification System which has the unique Auto-Blend method.

Details:

The protein chemist's main goal is to enhance the separation based on many principles to acheive a highly purified protein well resolved from the sample matrix. The biological sample may vary in resolution and recovery depending on many variables; pH, buffer, packing material, salts and samples themselves. Manipulation and control of these variables may improve the resolution and/or recovery of the proteins of interest. The Waters Auto-Blend method allows a convenient way to scout a variety of pH's which make the purification process easier and reproducible. The 650 system allows accurate blending of four buffers and allow a variety of pH's to be produced in a reproducible manner. Using stock buffers, the pH is chosen by the % of acidic buffer in the A line (100mM Tris-HCl) and basic buffer in the B line (100mM Tris Base). The desired salt concentration in the gradient is managed by blending salt from the C line (1M NaCl) and water in the D line. In methods development, one can easily scan a wide variety of pH's by programming the 650 system to blend different proportions of acidic and basic buffers. Combination of a unique ion-exchange packing to allow high capacity, resolution and recovery with Waters Auto-Blend to allow automated methods development makes an attractive package for the protein chemist. In this particular study, varying the pH form 7.7 to 8.6 significantly changes the elution profile of the enzyme LDH and the degree of purification is increased five fold in less than one day. Since there is no difference in recovery, the chemist can choose the best resolution which is defined at the conditions which give the highest specific activity. Quantitative recovery of the applied LDH activity was obtained at both pH 7.7 and 8.6.

System:

Waters 650 Advanced Protein Purification System, Waters Fraction Collector, and 484 Tunable UV/Visible Absorbance Detector.

References:

Auto-Blend

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Valving and Auto-Blend

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