biochrom
Expertise you can rely on

EZ Nin Reagent

EZ Nin Reagent™ is ready to use with superior stability and longevity for consistently excellent analysis of amino acids*

Application Note: Improved Formulation of Ninhydrin Reagent for Automated Amino Acid Analysis

J-P Veyssier, Biochrom Ltd, United Kingdom. Dr. M. Henderson, J. Mitchell & I. Sherratt, The Leeds Teaching Hospitals NHS Trust, United Kingdom.

Introduction: The importance of ninhydrin performance

Clinical scientists require the highest degree of certainty when diagnosing and monitoring patients with life-threatening disorders of amino acid metabolism. Ion exchange chromatography with post-column derivatization of amino acids is the gold standard in the field. The method is essential in performing confirmatory diagnoses of Inborn Errors of Metabolism, as well as accurate and reliable patient monitoring. Although this method is widely used and relied upon, it is dependent on the correct preparation of a key reagent: Ninhydrin. Unfortunately, Ninhydrin solutions are notoriously unstable with a limited lifespan of 4 weeks once prepared. Variability can be introduced during the preparation of the solution further increasing the potential error of analysis.

As the leading provider of amino acid analyzers, Biochrom understands the limitations of the current Ninhydrin solution. New EZ Nin Reagent™ from Biochrom is ready to use straight from the bottle, thereby eliminating variation from preparation. Ready to use EZ Nin Reagent is also insensitive to the effects of oxygen and light so it can be used on the instrument for up to one year without a decrease in measurement performance. An important benefit is that users can simply top up the reagent on the instrument for overnight and weekend analyses resulting in reduced chemical waste.

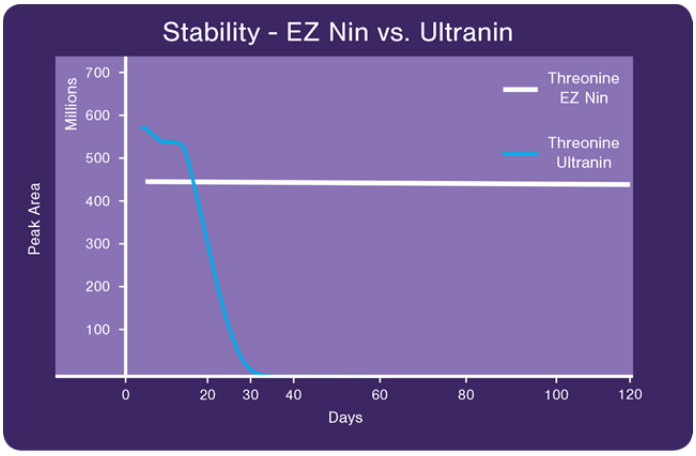
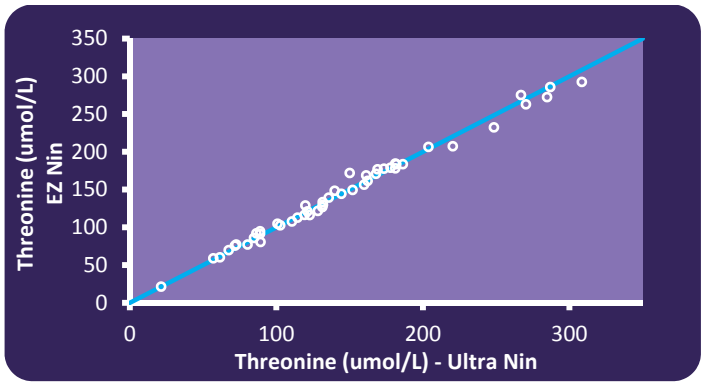


Figure 1.
Peak area is unchanged over 120 days using EZ Nin.
The peak area of Threonine analysed on a Bio30+ instrument using EZ Nin Reagent was measured at regular intervals over a period of 120 days. It showed a maximum variation of 3.2%RSD. In comparison, Ultra Nin solution gradually lost 75% of its sensitivity after 22 days and subsequently became unusable.

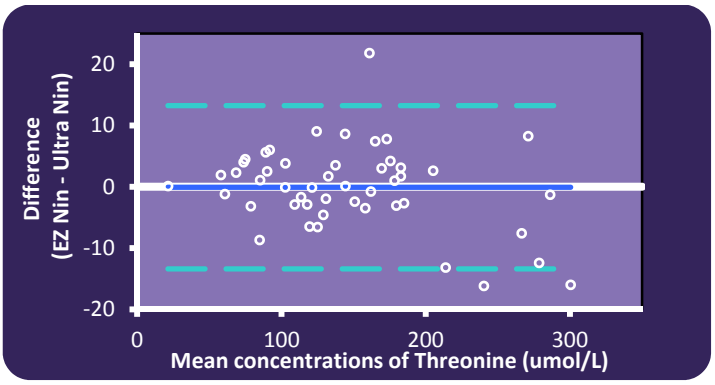
Methods and Results

EZ Nin Reagent was tested in parallel with Ultra Ninhydrin at the Department of Specialised Laboratory Medicine at St James Hospital, Leeds, United Kingdom. Anonymized, paired sets of patient samples of urine, plasma and CSF were measured in parallel using two Biochrom Amino Acid Analysers. Both instruments were optimized using an identical analysis program. The reaction coil of the instrument running EZ Nin Reagent was set at a slightly higher temperature of 138°C (as opposed to 135°C). The comparison occurred over four months resulting in the analysis of more than 100 patient samples.

Figure 2. Comparison of Threonine concentrations using EZ Nin vs. Ultra Ninhydrin



Comparison of Threonine concentrations obtained using EZ Nin vs. Ultra Nin.



Concentration bias observed (blue line) comparing concentrations obtained with EZ Nin vs. Ultra Nin. The dashed lines show the 95% confidence interval.

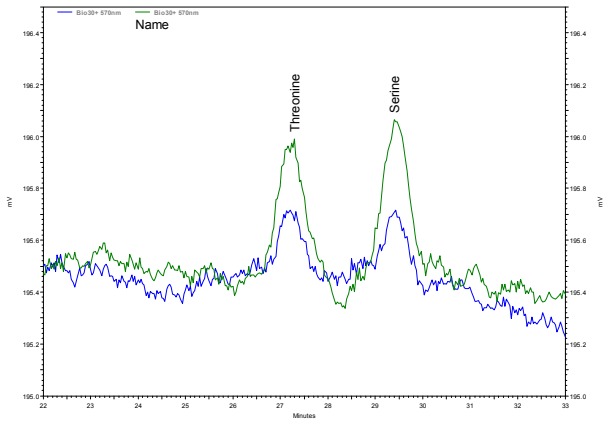
EZ Nin Reagent is a ninhydrin formulation for use in ion exchange chromatography systems with post column derivatization.

The samples were prepared according to the standard deproteinisation method using 5-sulfosalicylic acid solution, followed by centrifugation at 10,000rpm for 5 minutes. 50µL aliquots of the supernatant were injected into both analysers. The paired samples were analysed using the Lithium High Resolution programme on both instruments. Each Biochrom unit was calibrated using the published internal standard calculation method with Norleucine.
The concentrations of 7 analytes (Thr, Citr, Val, Leu, Phe, Lys & Arg) were compared using the Bland Altman statistical test of identity. Excellent correlation was obtained. The bias measured was as low as 0.09µmol/L (Threonine) *(Figure 2)*. The largest bias obtained was 11µmol/L (Leucine).

Amino Acids	R^2	Amino Acids	R^2
Phosphoserine	1.0000	Valine	0.9999
Taurine	1.0000	Methionine	1.0000
Phosphoethanolamine	1.0000	Isoleucine	1.0000
Aspartic Acid	1.0000	Leucine	1.0000
Threonine	1.0000	Tyrosine	1.0000
Serine	1.0000	Phenylalanine	1.0000
Asparagine	1.0000	β Aminoisobutyric Acid	0.9997
Glutamic Acid	1.0000	γ Aminoisobutyric Acid	0.9997
α Amino Adipic Acid	1.0000	Ornithine	1.0000
Glycine	1.0000	Lysine	1.0000
Alanine	1.0000	1-Methyl Histidine	1.0000
Citrulline	1.0000	Histidine	1.0000
3-Methyl histidine	1.0000	Arginine	1.0000

Figure 3.
EZ Nin demonstrates excellent linearity in comparing 26 analytes.

Linearity was calculated for 26 analytes for concentrations from 50 picomoles to 10 nanomoles (2.5µmol/L and 500µmol/L with an injection volume of 20µL of Sigma Aldrich amino acid standards (A6407 & A6282). Excellent linearity was obtained for all amino acids with a correlation coefficient r^2>0.9997.



Threonine and Serine peaks – 10 picomoles, S/N>3 (blue trace) and 50 picomoles (green trace).

Figure 4.
Limit of detection and limit of quantification using EZ Nin Reagent.

20µL of two standard solutions of amino acids were injected at a concentration of 0.5µmol/L and 2.5µmol/L respectively (10 picomoles and 50 picomoles on column) and run using the Lithium High Performance programme with the reaction coil set at a temperature of 138°C. The LOD for Threonine and Serine was determined as being 10 picomoles (S/N ratio>3). The Limit of Quantification was determined as being 50 picomoles (S/N ratio>10).

Amino Acid	S/N Ratio at 10 picomoles	S/N Ratio at 50 picomoles
Threonine	3.7	9.5
Serine	4.1	10.3

Conclusion: EZ Nin Reagent™ sets a superior standard in Amino Acid Analysis

EZ Nin Reagent proves to be an excellent alternative to the traditional Ninhydrin formulation in key specifications such as stability, amino acid concentration determinations in paired patient samples, linearity and limit of detection (10 pmol).

For more information on application notes and ordering, please contact your local Sales representative or visit our website by scanning the QR code