



TRIONE® Ninhydrin Reagent

A Prepared Reagent for Automated Post-column Derivatization of Primary and Secondary Amines

TRIONE Ninhydrin reagent is specially formulated for amino acid analysis. It is so stable that it does not require refrigeration, either in shipment, storage, or in the reservoir. Quantitation is consistent from the first to the last mL, so there is no waste. The high signal-to-noise ratio of TRIONE, when compared to DMSO-containing reagents, permits detection sensitivity to be increased with minimum increase in background noise — a feature particularly appreciated at sample concentrations of <50 picomoles.

Two preparations are available to suit your usage and storage requirements:

T100

- Pour into your reservoir and use; the ultimate in convenience with a minimum of handling.
- Three-month shelf life.

T200

- Combine two solutions, swirl, and use.
- Twelve-month shelf life before mixing; one month in the reservoir.



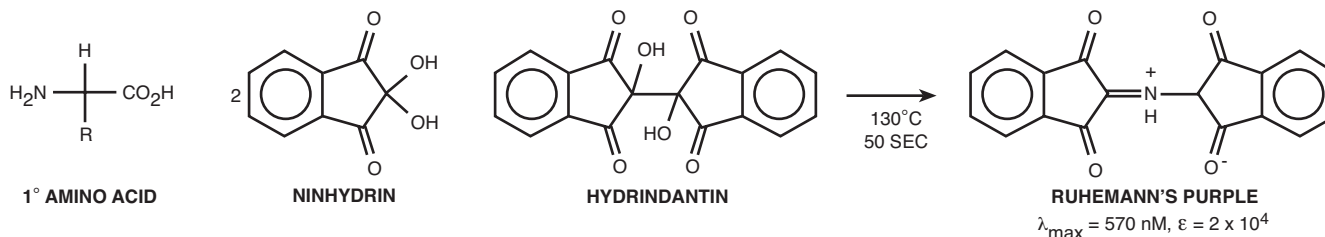
TRIONE Ninhydrin Reagent is a proprietary formulation containing Ninhydrin, Hydrindantin (reduced Ninhydrin), a Lithium Acetate buffer, and Sulfolane, a water-miscible organic solvent. The solvent is necessary to maintain the solubility of both the Hydrindantin and the primary amine product, Ruhemann's Purple. The buffer is required because the reaction is pH dependent. The active ingredients — Ninhydrin and Hydrindantin — are required for proper development of secondary and primary amines, respectively.

Ninhydrin is a selective oxidizing agent which causes oxidative decarboxylation of α -amino acids, producing CO_2 , NH_3 , and an aldehyde with one less

carbon atom than the parent acid. The reduced ninhydrin then reacts with the liberated Ammonia to form Ruhemann's Purple, a complex which maximally absorbs light at 570 mn. Secondary amines, Proline and 4-Hydroxyproline, react via a different path and form a



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yellow derivative with an optimal absorbance at 440 nm.

Since the reaction with amines is highly specific and the absorption characteristics of the formed chromophores follow Beer's Law, reagents based on Ninhydrin have long been the most popular choice for detection and quantitation of amines and amino acids.

Ninhydrin reacts slowly at room temperature. Consequently, in automated amino acid analysis, elevated temperatures of up to 130°C are employed to reduce the conversion time to about one minute. The reduction in dwell time results in minimal band spreading with no sacrifice in reproducibility.

Detection of the derivatives can be accomplished with variable wavelength detectors or with fixed filter photometers. Background subtraction at 690-700 nm is sometimes used to improve signal-to-noise.

TRIONE AGING

The three-month aging process for TRIONE begins when it is made.

On storage an unopened bottle of **TRIONE T100** does not lose potency. Rather, signal-to-noise improves approximately 0.1% per day. This is due to a change in the Hydrindantin. The changed form of Hydrindantin is less soluble and therefore is more likely to precipitate in the heated reactor.

TRIONE T200 can be stored unopened for 12 months without change.

TRIONE OXIDATION

Upon exposure to air (O₂) TRIONE can become oxidized, thereby losing potency for primary amines. Depending on the exposure, part of or all of the Hydrindantin will be oxidized to Ninhydrin. Only primary amine development requires Hydrindantin. Consequently, as the Hydrindantin disappears, so will the primary amine signal.

To reduce the potential for oxidation it is of critical importance not only to maintain the reservoir under N₂, but also to ensure that only air-impermeable tubing is connected between

the gas regulator and reservoir, and between the reagent pump and reservoir. Either Saran® or PEEK may be used, with Saran the more cost-effective alternative. Saran has approximately 1/1000 the oxygen permeability of Teflon® or other fluorocarbon tubing.

Secondary amine development requires no Hydrindantin, so upon oxidation no change in signal occurs for secondary amines, e.g., Proline and 4-Hydroxyproline. However, since the background color of TRIONE is proportional to the Hydrindantin concentration (λ max = approx. 400nm), and the secondary amine absorption is near (λ max = 440 nm), the loss of primary signal makes the secondary signal appear greater than usual for oxidized TRIONE.

STORAGE AND HANDLING

Both TRIONE T100 and T200 Ninhydrin reagents are sensitive to oxygen. They should be stored at room temperature (20-25°C) in the original, unopened containers.

T100 is guaranteed to be usable for three months from the time it is manufactured.

In some automated amino acid analyzers such as the Beckman System 6300, refrigeration during storage and use of the Beckman Nin-RX reagent is mandatory. TRIONE may be employed under refrigeration in the System 6300 as long as it is maintained above 5°C. If it is necessary to keep the samples at a lower temperature, then the Ninhydrin reservoir should be placed in the pump compartment of the analyzer.

The bottles in which TRIONE T100 and T200 are shipped are not pressure-rated, and must not be used as a reservoir. We recommend installing the Pickering Reservoir Assembly, which can be pressurized safely to 0.15-0.3 bar with Nitrogen.

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