

➔ TRIONE® NINHYDRIN REAGENT

A PREPARED REAGENT FOR 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 mL to the last, so there is no waste. The high signal-to-noise ratio of TRIONE (especially 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

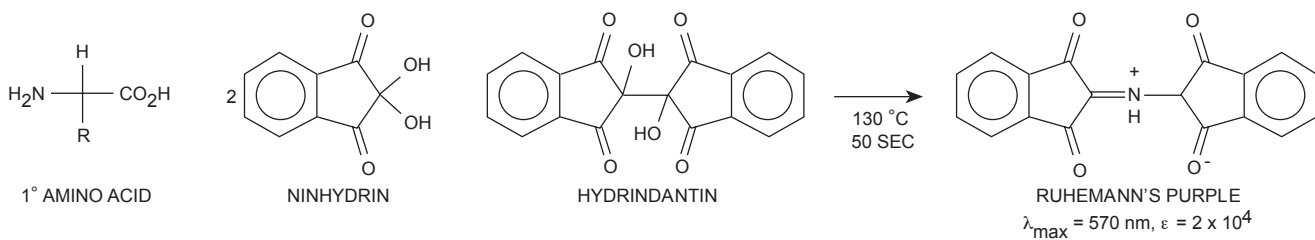
- Pour into your reservoir and use; the ultimate in convenience with minimum handling.
- Four-month shelf life.

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 nm. Secondary amines, such as Proline and 4-Hydroxyproline, react via a different path and form a 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 without sacrificing 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 four-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 – an important reason not to use expired TRIONE. 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.

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. 400 nm), 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 four months from the time it is manufactured.

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.

TRIONE (U.S. Patent no. 4,274,833) is a registered trademark of Pickering Laboratories, Inc.



1280 Space Park Way / Mountain View, CA 94043
sales@pickeringlabs.com / support@pickeringlabs.com
800-654-3330 / 650-694-6700 / Fax: 650-968-0749
RB12-D