



Ammonia (K-LARGE) Procedure for ChemWell®-T Auto-Analyser

Requirements:

- L-Arginine/Urea/Ammonia Assay Kit (K-LARGE) (provides ~ 600 assays).
- K-LARGE (AMMONIA) ChemWell®-T assay file.
- Use in association with the L-Arginine/Urea/Ammonia Assay Kit (K-LARGE) product data booklet.

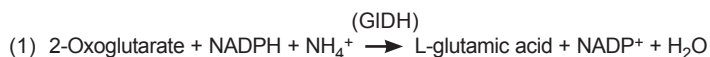
Use:

For the specific measurement of ammonia especially in wines, fruit juices, beverages and food products.

For specific sample preparation methods refer to the L-Arginine/Urea/Ammonia Assay Kit (K-LARGE) data booklet.

Assay Principle:

Conversion of ammonia (as ammonium ions) via the following reaction is directly proportional to the coupled consumption of NADPH:



Procedure:

Prepare the assay reagents and calibrators and use with the K-LARGE (AMMONIA) ChemWell®-T assay file.

L-Arginine/Urea/Ammonia Assay Kit Components:

- Bottle 1:** Buffer (18 mL, pH 8.0) plus 2-oxoglutarate and sodium azide (0.02% w/v) as a preservative. Stable for > 2 years at 4°C.
- Bottle 2:** NADPH. Stable for > 5 years at -20°C.
- Bottle 3:** Glutamate dehydrogenase suspension (1.1 mL). Stable for > 2 years at 4°C.

Preparation of Kit Components:

1. Use the contents of bottle 1 as supplied. Stable for > 2 years at 4°C.
2. Dissolve the contents of bottle 2 in 12 mL of distilled water. **Stable for > 1 year at 4°C** or > 2 years at -20°C (to avoid repetitive freeze / thaw cycles, divide into appropriately sized aliquots and store in polypropylene tubes).
3. Use the contents of bottle 3 as supplied. Swirl the bottle to mix contents before use. Stable for > 2 years at 4°C.

Preparation of Assay Reagents: (per ~ 100 assays)

Reagent 1:

Component	Volume
distilled water	15 mL
bottle 1 (buffer)	3 mL
*bottle 2 (NADPH)	2 mL
Total volume	20 mL

*after adding 12 mL of distilled water

Reagent 1 stability: > 7 days at 4°C

Reagent 2:

Component	Volume
distilled water	2.4 mL
bottle 3 (GIDH)	0.18 mL
Total volume	2.58 mL

Reagent 2 stability: > 7 days at 4°C

Calibrators:

- K-LARGE Ammonia 1: 0 g/L (use distilled water)
K-LARGE Ammonia 2: 0.015 g/L ammonia
K-LARGE Ammonia 3: 0.03 g/L ammonia
K-LARGE Ammonia 4: 0.06 g/L ammonia

Assay Parameters:

- Assay volumes: Reagent 1: 0.200 mL
Sample: 0.010 mL
Reagent 2: 0.025 mL
- Calibrators: 0, 0.015, 0.03, 0.06 g/L ammonia
Reaction time: 5 min at 37°C
Wavelength: 340 nm
Assay type: endpoint
Reaction direction: decrease
Linearity: up to 0.06 g/L of ammonia

