



# Urea (K-LARGE)

## Procedure for ChemWell®-T Auto-Analyser

### Requirements:

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

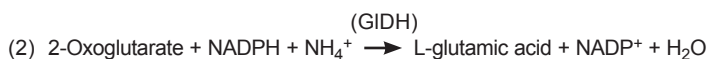
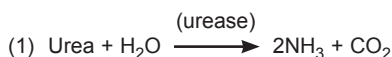
### Use:

For the specific measurement of urea 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 urea via the following reactions is directly proportional to the coupled consumption of NADPH:



### Procedure:

Prepare the assay reagents and calibrators and use with the K-LARGE (UREA) 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.

**Bottle 4:** Urease solution (2.7 mL). Stable for > 2 years at -20°C.

### Preparation of Kit Components:

- 1 & 3.** Use the contents of bottles 1 and 3 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).
- 4.** Use the contents of bottle 4 as supplied. Stable for > 2 years at -20°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

#### Reagent 3:

Component	Volume
distilled water	2.1 mL
bottle 4 (urease)	0.45 mL
Total volume	2.55 mL

Reagent 3 stability: > 2 days at 4°C

### Calibrators:

- K-LARGE Urea 1: 0 g/L (use distilled water)
- K-LARGE Urea 2: 0.025 g/L urea
- K-LARGE Urea 3: 0.05 g/L urea
- K-LARGE Urea 4: 0.1 g/L urea

### Assay Parameters:

Assay volumes: Reagent 1: 0.200 mL  
Sample: 0.010 mL  
Reagent 2: 0.025 mL  
Reagent 3: 0.025 mL

Calibrators: 0, 0.025, 0.05, 0.1 g/L urea  
Reaction time: 5 min at 37°C plus 6 min at 37°C  
Wavelength: 340 nm  
Assay type: endpoint  
Reaction direction: decrease  
Linearity: up to 0.1 g/L of urea

