



6330 Nancy Ridge Drive, Suite 103  
San Diego, CA 92121  
Tel: (858) 450-0048

### Chloride Enzymatic Assay Kit

Catalog Number: BQ 006 - EAEL

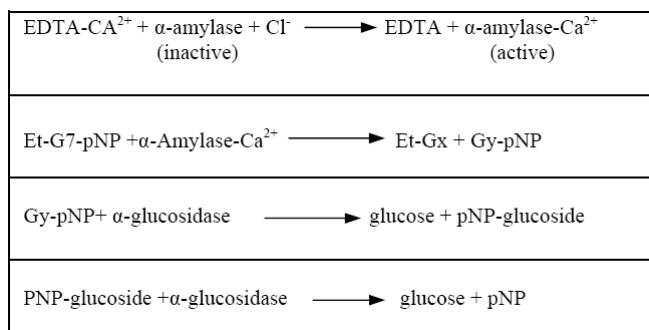
Wavelength: 405 nm  
Linear range: 70-140 mM

#### Intended Use

For quantitative determination of chloride in human serum. The determination of chloride in serum is most commonly performed for the diagnosis of proper hydration, osmotic pressure, and acid/base equilibrium. Elevated serum chloride values may be seen in dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary obstruction. Low serum chloride values are found with extensive burns, excessive vomiting, intestinal obstruction, nephritis, and metabolic acidosis.<sup>1,2</sup>

#### Assay Principle

Mammalian  $\alpha$ -amylase, which normally involves binding with calcium ion, is deactivated by removing calcium ion by adding a high concentration of EDTA in the absence of chloride anion. The deactivated  $\alpha$ -amylase is reactivated by addition of chloride anion, which allows the calcium ion to re-associate with the enzyme.<sup>3</sup> The reactivation of  $\alpha$ -amylase activity is proportional to the concentration of chloride anion present. Ethylidene blocked p-nitrophenyl-maltoheptaoside (EPS-G7) is used as the substrate.<sup>4</sup> Reactivated  $\alpha$ -amylase hydrolyzes EPS-G7 to Et-Gx and Gy-pNP. Gy-pNP is further hydrolyzed by a coupled enzyme,  $\alpha$ -glucosidase to glucose and pNP which is quantitated colorimetrically at 405 nm. The amount of pNP formed is directly proportional to the  $\alpha$ -amylase activity in the sample.



G = glucose;  
x and y = 2-5 and x + y = 7  
pNP = p-nitrophenol

#### Reagent Table (200 tests)

R1	Substrate	
	EPS-G7	1 x 45 mL
R2	Enzymes	1 x 9 mL
Low Calibrator		1 x 2 mL
High Calibrator		1 x 2 mL

#### Reagent Preparation

R1 and R2 are ready-to-use liquid stable reagents.

#### Low and high calibrators

Low and high calibrators included are ready to use and are stable up to expiration date when stored at 2 – 8 °C.

#### Specimen Collection and Handling

The Bio-Quant Chloride Enzymatic Assay is formulated for use with non-haemolysed serum treated with heparin or EDTA-Na. No special handling or pretreatment is needed. Use serum that has been separated from the blood clot immediately after drawing. Avoid contamination of blood with tissue fluid.

Chloride is stable in serum for one day at room temperature, up to one week at 4 °C and for three months under frozen when stored tightly capped.

**Note:** Serum specimens and all materials coming in contact with them should be handled and disposed as if capable of transmitting infection. Avoid contact with skin by wearing gloves and proper laboratory attire.

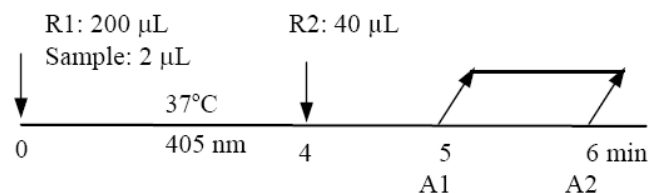
#### Expected Chloride values

Serum: 98-106 mM.<sup>1</sup>

It is recommended that each laboratory establish its own range of expected values.

#### Assay Procedure (Manual)

1. Zero the spectrophotometer at 405 nm against water.
2. To a cuvette is added 200  $\mu\text{L}$  of R1, and 2  $\mu\text{L}$  of sample. Mix well immediately. After incubation for 4 min, Add 40  $\mu\text{L}$  of R2.
3. Read absorbance (405 nm) at 1 minute after addition of R2 as A1. Incubate for additional 1 minute and read the absorbance as A2.
4. Calculate  $\Delta A = A2 - A1$



#### Calibration

1. This assay should be calibrated daily using the enclosed low and high calibrators.
2. Construct a calibration curve by plotting the  $\Delta A$  values of the calibrators against the corresponding concentrations.
3. The Chloride concentration of the sample is read from the calibration curve.

#### Assay Procedure (Auto)

See appropriate instrument application instructions. The following is HITACHI parameters.

HITACHI 7170 Parameters	Amylase BQ006EAEL
Test	Chloride
Assay Code	2 point
Assay Point	(30)-(35)**
Wavelength	700/405
Calibration Method	Linear
Unit	mmol/L
Sample volume	(2)(2)
Reagent vol. R1	(200)(20)(NO)
Reagent vol. R2	(40)(20)(NO)
Std (1)	(70)
Std (2)	(120)
ABS Limit	(32000)(Increase)
Prozone limit	(0) (lower)
Expected value	(-999)-(32000)
Tech. Limit	(-999)-(32000)

\*\* Each reading cycle is 12 seconds.

**Quality Control**

Good laboratory practice recommends the use of control materials. Users should follow the appropriate federal, state and local guideline concerning the running of external quality control. To ensure adequate quality control, normal and abnormal control with known values should be run as unknown samples.

**Interferences**

Bilirubin up to 200 mg/L and hemoglobin up to 5000 mg/L gave no interference. Bromide can cause falsely elevated chloride values. Assay response of fluoride and iodide was 0%.

**Limitations of the Procedure**

The assay is designed for use with human serum sample only. Linearity is 70-140 mM. Samples that exceeded the linearity limit (140 mM) should be diluted with an equal volume of water. Multiply the result by two. Hydrochloric acid fumes may cause high results. There is a possibility that technical or procedural errors as well as other substances factors not listed may interfere with the test.

**References**

1. Tietz, N.W.: Fundamental of clinical chemistry, W.B. Saunders, Philadelphia, PA, p897 (1976)
2. White, W.L., et al., Chemistry for technologist, 3rd Ed, The C.V. Mosby Co, St. Louis, p182 (1970)
3. Ono, T., et al., Clin. Chem. 34: 552-553 (1988)
4. Klaus Lorentz. Clin. Chem. 46(5) 644-649 (2000)