BECKMAN COULTER CE

AU

Instructions For Use

Alpha Amylase
a-Amylase

REF OSR6006 4 x 10 mL R1
OSR6106 4 x 40 mL R1

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Kinetic colour test for the quantitative determination of α-amylase, [1,4-α-D-glucan 4-glucanohydrolase, EC 3.2.1.1], in human serum, plasma and urine on Beckman Coulter analysers.

SUMMARY AND EXPLANATION

Reference[1,2,3]

Amylases are a group of hydrolases that split complex carbohydrates composed of alpha-D-glucose units linked through carbon atoms 1 and 4 located on adjacent glucose residues. In the body, amylase is present in a number of organs and tissues. The greatest concentration is present in the pancreas, where the enzyme is synthesised by the acinar cells and then secreted into the intestinal tract by way of the pancreatic duct system. The salivary glands also secrete a potent amylase to initiate hydrolysis of starches while the food is still in the mouth and oesophagus.

Diseases resulting in elevation of plasma alpha-amylase include: acute pancreatitis, parotitis, alcoholism, renal insufficiency and diseases such as viral hepatitis, AIDS, abdominal typhoid, sarcoidosis and trauma to the upper abdomen. There is also a detectable increase in amylase after an ERCP procedure.

In acute pancreatitis, amylase increases 5-6 hours after the onset of symptoms and remains elevated for 2-5 days. The increase in plasma activity does not reflect disease severity and conversely, extensive destruction of the pancreas may not cause a significant increase in the plasma concentration of pancreatic alpha-amylase.

Alpha amylase is excreted by glomerular filtration and then 50% of it is reabsorbed by the tubules. This reabsorption is significantly reduced in transient tubular damage, after burns, in the presence of diabetic ketoacidosis and acute pancreatitis as well as proteinuria resulting in an increase of alpha-amylase clearance. The measurement of alpha amylase in urine is indicated in the investigation of hyperamylasemia associated with macroamylasemia or renal insufficiency. Hypoamylasemia has been observed in advanced cystic fibrosis, severe liver disease and pancreatectomy. Also due to the decreased concentration of the salivary fraction hypoamylasemia has been found in obese subjects.

METHODOLOGY

Reference[4]

The α-amylase colour test employs 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) as substrate. This substrate reacts directly with α-amylase and does not require the presence of ancillary enzymes. The release of 2-chloro-4-nitrophenol (CNP) from the substrate and the resulting absorbance increase at 410 nm is directly proportional to the α-amylase activity in the sample.

CHEMICAL REACTION SCHEME

$$CNPG_3 + H_2O \xrightarrow{\alpha\text{-amylase}} CNP + Maltotriose$$

SPECIMEN

TYPE OF SPECIMEN

Serum, heparinised plasma. Haemolysed and strongly icteric samples should be avoided, separate from blood cells as soon as possible.

Stable in serum and plasma for 7 days when stored at 2…25°C.[5]

Plasma using EDTA, oxalate and citrate should be avoided.[1,6]

Urine: Timed or random sample.[1] Adjust pH to approximately 7.0 before storage.[6]

Stable in urine for 10 days when stored at 2…8°C and 2 days when stored at 15…25°C[5]

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.
Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

MES (pH 6.05)	36.1 mmol/L
Calcium acetate	3.60 mmol/L
NaCl	37.2 mmol/L
Potassium thiocyanate	253 mmol/L
$CNPG_3$	1.63 mmol/L
Preservatives	

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

The reagent is ready for use and can be placed directly on board the instrument.

Care should be taken when handling this reagent to avoid contamination with skin and body fluids.

STORAGE AND STABILITY

The reagent is stable, unopened, up to the stated expiry date when stored at 2…8°C. Once open the reagent stored on board the instrument is stable for 30 days.

Discard reagents if any discolouration is observed.

CALIBRATION

CALIBRATION INFORMATION

The test is run in MB-mode. To provide a robust approach to generate the analyser specific MB factor, it is recommended that 5 separate calibration events should be used. A fresh vial of calibrator, utilising System Calibrator Cat No. 66300 for serum application and Urine Calibrator Cat no B64606, in the AB calibration mode, should be used for each of these runs. When calculating the mean factor from the separate runs the data should be examined for obvious outliers which should be repeated and replaced. For the AU2700/AU5400 this procedure needs to be performed for each ring. Quality control procedures should be undertaken immediately following calibration in accordance with good laboratory practice.

The calibrator values are traceable to a Beckman Coulter master calibrator.

Re-establishment of the analyser specific MB factor is recommended when a critical part of the analyser is replaced.

Reagent blank measurement is recommended when changing to a new lot of reagent.

QUALITY CONTROL

Controls Cat. No. ODC0003 and ODC0004 or other control materials with values determined by this Beckman Coulter system may be used for the serum/plasma application.

Biorad Liquichek Urine Chemistry Controls Cat. No. 397 and 398 or other control materials with values determined by this Beckman Coulter system may be used for the urine application.

Each laboratory should establish its own control frequency.

Good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration is performed. Values obtained for the controls should fall within specified limits as defined by the user. If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement.

CALCULATIONS

The Beckman Coulter analysers automatically compute the α-amylase activity of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Serum/Plasma[3]	22 – 80 U/L (0.36 – 1.33 µkat/L)
Urine[7]	42 – 321 U/L (0.7 – 5.35 µkat/L)

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

INTERFERENCES

Results of serum studies conducted to evaluate the susceptibility of the method to interference were as follows:

Icterus:	Interference less than 10% up to 20 mg/dL or 342 µmol/L bilirubin
Haemolysis:	Interference less than 10% up to 2.5 g/L haemoglobin
Lipemia:	Interference less than 5% up to 1,000 mg/dL Intralipid.

Eltrombopag and its metabolites may interfere with this assay causing erroneously low patient results.

Results of urine studies conducted to evaluate the susceptibility of the method to interference were as follows:

Ascorbate:	Interference less than 10% up to 50 mg/dL ascorbate
Icterus:	Interference less than 10% up to 40 mg/dL or 684 µmol/L bilirubin
Haemolysis:	Interference less than 5% up to 5 g/L haemoglobin

Refer to Young[8] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within an enzyme activity range of 10 – 2,000 U/L (0.2 – 33.3 µkat/L) for serum and plasma. The test is linear within an enzyme activity range of 5 – 4,800 U/L (0.1 – 80 µkat/L) for urine.

SENSITIVITY

The lowest detectable level using serum settings on an AU5800 analyser was calculated as 1 U/L.

The lowest detectable level using urine settings on an AU2700 analyser was calculated as 2 U/L.

The lowest detectable level represents the lowest measurable level of α-amylase that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

METHODS COMPARISON

Patient serum samples were used to compare this assay on the AU5800 against the AU2700. Results of linear regression analysis were as follows:

y = 1.035x - 2.498	r = 1.000	n = 106	Sample range = 12 - 1889 U/L

Patient urine samples were used to compare this α-Amylase OSR6106 assay on the AU2700 against another commercially available α-amylase assay. Results of linear regression analysis were as follows:

y = 1.067x – 4.492	r = 0.995	n = 125	Sample range = 18 – 339 U/L

PRECISION

The following data was obtained on an AU5800 using 3 serum pools analysed over 20 days.

n = 80	**Within-run**		**Total**	
Mean U/L	**SD**	**CV%**	**SD**	**CV%**
50	0.7	1.4	0.93	1.9
140	1.53	1.1	2.69	1.9
1533	18.78	1.2	28.12	1.8

The following data was obtained on an AU640 using 3 urine pools analysed over 20 days.

n = 80	**Within-run**		**Total**	
Mean U/L	**SD**	**CV%**	**SD**	**CV%**
45.97	0.69	1.51	2.13	4.64
494.94	3.99	0.81	14.1	2.85
3207.84	50.73	1.58	101.63	3.17

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	**Description**
AMY1N	Amylase (Serum)
AMY1N	Amylase (Urine)

Setting Sheet Footnotes

User defined

* Values set for working in U/L. To work in SI units (µkat/L) divide by 60.

§ For use in AB mode only, refer to leaflet for further instruction.

REVISION HISTORY

Added new languages

Preceding version revision history

Revised GHS section

Revised Interferences section.

REFERENCES

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3. Moss DW, Henderson RA. Clinical Enzymology. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry. Philadelphia:WB Saunders Company, 1999;689-698.

4. IFCC methods for measurement of catalytic concentration of enzymes. Part 9. IFCC method for alpha-amylase [1,4-alpha-D-glucan 4-glucanohydrolase, EC 3.2.1.1]. International Federation of Clinical Chemistry. Clin Chim Acta. 1999;281(1-2):S5-39.

5. Ehret W, Heil W, Schmitt Y, Töpfer G, Wisser H, Zawta B, et al. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. WHO/DIL/LAB/99.1 Rev.2:22, 46pp.

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