Amylase EPS-G7 Reagent
for Beckman Coulter™ SYNCHRON® and UniCel® Systems‡

REF A45288 (2 x 200 tests)

INTENDED USE
Reagent for the quantitative determination of α-Amylase (1,4-α-D-glucan glucanohydrolase EC3.2.1.1) in human serum, plasma or urine on Beckman Coulter Synchron CX/LX and UniCel DxC Systems.

CLINICAL SIGNIFICANCE[1,2]
α-Amylase catalyses the hydrolysis of 1,4 – α-glucosidic linkages in polysaccharides to produce maltose and other oligosaccharides. The enzyme is a relatively small molecule which is rapidly cleared by the kidneys and excreted in the urine. The enzyme present in the serum and urine is predominantly of pancreatic (P-AMY) and salivary (S-AMY) origin.
α-Amylase is most frequently measured in the diagnosis of acute pancreatitis, when serum levels may be grossly elevated (a four to six fold elevation above the upper reference limit is usual). In acute pancreatitis α- Amylase levels start to rise 5 to 8 hours after the onset of symptoms, reach a peak between 12 and 72 hours and return to normal levels by the 3rd or 4th day.

METHODOLOGY[2,3]
Amylase methods that utilise well defined substrates with short glucosyl chains offer significant benefits over amyloclastic and saccharogenic procedures and, as a result, have gained wide acceptance. One such substrate, ethylidene–pNP-G7 (E-pNP-G7), also commonly referred to as EPS, is utilised in this method. The use of the ethylidene prevents exo – enzymes from breaking down the substrate, so in the absence of α-Amylase no color change is observed. α-Amylase present in the sample cleaves the substrate releasing smaller fragments that are acted upon by α-glucosidase, causing the ultimate release of the chromophore.
The series of reactions involved in this assay system are as follows:

1. $EPS + H_2O \xrightarrow{\alpha\text{-}amylase} 4,6\text{-Ethylidene-G x} + 4\text{-Nitrophenyl-G (7-x)}$

2. $4\text{-Nitrophenyl -G (7-x)} + (7\text{-x})\ H_2O \xrightarrow{\alpha\text{-}glucosidase} (7\text{-x})\ \text{Glucose} + 4\text{-Nitrophenoxide}$

The rate of formation of 4-Nitrophenoxide is proportional to the α-Amylase present in the sample and is measured by the rate of increase in absorbance at 410 nm (secondary wavelength of 560 nm) on the Beckman Coulter Synchron LX/CX and Unicel DxC Systems. When run with approved System Parameters, Amylase EPS-G7 Reagent will recover IFCC Amylase values.

REAGENT COMPOSITION

Active Ingredients	**Concentration**
Reagent A(Compartment A)	
α-Glucosidase (microbial)	>9700 U/L
NaCl	87 mmol/L
$MgCl_2$	12.6 mmol/L
CaCl	0.08 mmol/L
Buffer	53.3 mmol/L
Preservative	
pH 7.2 ± 0.05 at 20°C.	
Reagent B (Compartment B)	
EPS	22 mmol/L
Buffer	54.4 mmol/L
Preservative	
pH 7.2 ± 0.05 at 20°C.	

WARNING: Amylase EPS-G7 is for in vitro diagnostic use only. Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Amylase EPS-G7 Reagent for Beckman Coulter Synchron CX/LX and UniCel DxC Systems Material Safety Data Sheet.

REAGENT PREPARATION
Reagent is supplied ready to use. Transfer the contents of Reagent A and Reagent B into appropriate compartments of the user Defined Cartridge included in the kit as shown in the table below. Use care to avoid contamination.

Amylase EPS-G7 Kit	Compartment A	Compartment B
Reagent A	40 mL	–
Reagent B	-	8.5 mL

STABILITY AND STORAGE
The unopened reagents are stable until the expiration date when stored at 2-8°C. When stored on Synchron LX/CX and UniCel DxC Systems, the reagent is stable for 35 days.

Indications of Reagent Deterioration:
- Turbidity
- Failure to recover control values within the assigned range
- "BL ABS HI" flag.

SPECIMEN COLLECTION AND HANDLING
Serum: Use non-haemolysed serum.
Plasma: Li-heparin or Na-heparin.
Urine: Random or timed collections are valid specimens.[4]
Storage: α-Amylase is exceptionally stable and serum samples may be stored for at least 4 days at room temperature and for at least 2 weeks at 4°C.[2] Urine samples are stable for 7 days when stored at 4°C. If it is expected that there will be a delay in transporting the urine sample to the laboratory, the use of a chemical preservative such as Merthiolate (0.24 mmol/L) is recommended.[4]

MATERIALS PROVIDED
- Thermo Scientific Amylase EPS-G7 for Beckman Coulter Synchron LX/CX and UniCel DxC Systems.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- Beckman Coulter Synchron LX/CX and UniCel DxC chemistry analyzers.
- Beckman Coulter sample cups.
- Assayed Normal and Abnormal Controls.

TESTING PROCEDURES
Load the reagent onto the system as directed in the Operations Manual. Program samples and controls for analysis as directed in the Operations Manual.
For Synchron LX/CX and Unicel DxC System parameters refer to the System Parameters section of this package insert.

CALIBRATION
Calibration is not required. The Synchron LX/CX and UniCel DxC Systems calculates U/L of activity by multiplying the measured rate of reaction by the programmed Calculation Factor (Refer to the System Parameters Section of this package insert). The calculation factor has been derived to provide traceability to the IFCC Amylase reference measurement procedure.[3]

CALCULATIONS
Results are calculated automatically by the Synchron LX/CX and UniCel DxC Systems.
Unit conversion: U/L x 16.67 x 10^{-3} = µkat/L.

QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:
- At least once per day or as established by the laboratory.
- When a new reagent cartridge is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate that the assay may be out of control. The following corrective actions are recommended in such situations:
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS
1. Analytical specificity studies to determine the level of interference from various sample components were carried out on the CX and LX/DxC. No interference was observed below the following interferent concentration limits (pass criterion, initial control value ± 10%):

	LX/DxC	CX
Haemoglobin	900 mg/dL	1000 mg/dL
Lipaemia (using Intralipid)	2000 mg/dL	1000 mg/dL
Glucose	2160 mg/dL	2160 mg/dL
Free Bilirubin	60 mg/dL	60 mg/dL
Conjugated Bilirubin	60 mg/dL	60 mg/dL
Ascorbic Acid	200 mg/dL	200 mg/dL

EXPECTED VALUES[5]

Serum/Plasma:	At 37°C	28 - 100 U/L (0.468 - 1.67 µkat/L)
* Urine - Male	At 37°C	16 - 491 U/L (0.267 - 8.18 µkat/L)
* Urine - Female	At 37°C	21 - 447 U/L (0.350 - 7.45 µkat/L)

* Expected values for urine were calculated from spontaneously voided samples.

ADDITIONAL INFORMATION
Since Beckman Coulter does not manufacture the reagent or perform quality control or other tests on individual lots, Beckman Coulter cannot be responsible for the quality of the data obtained which is caused by performance of the reagent, any variation between lots of reagent, or protocol changes by the manufacturer.

SHIPPING DAMAGE
If damaged product is received, notify your Beckman Coulter Clinical Support Center.

PERFORMANCE DATA
The following data was obtained using Thermo Scientific Amylase EPS-G7 Reagent on the Beckman Coulter Synchron LX/CX and UniCel DxC Systems according to established procedures.

MEASURING RANGE
When run as recommended the measuring range of the assay with all recommended specimen matrices is as follows:

LX/DxC: 4 - 2000 U/L (0.067 - 33.3 µkat/L)
CX: 4 - 1800 U/L (0.067 - 30.0 µkat/L)

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LIMIT OF DETECTION

The limit of detection represents the lowest measurable analyte level that can be significantly distinguished from zero. It is calculated as the value lying two standard deviations above the mean estimate for an appropriate zero (blank) sample.
When run as recommended the limit of detection is:

LX/DxC: 4 U/L (0.067 µkat/L)
CX: 4 U/L (0.067 µkat/L)

IMPRECISION

Precision was evaluated using the NCCLS (CLSI) EP5-A2 guideline.[6] Studies to represent typical performance on a well maintained analyser were carried out at the same site over a period of 20 days (40 runs) using three levels of commercially available quality control serum and two levels of quality control urine. Two runs per day were carried out by the same operator on the same lots of reagent, on single CX and LX/DxC analyzers.

LX/DxC		Serum						Urine			
		Level 1		Level 2		Level 3		Level 1		Level 2	
		U/L	µkat/L	U/L	µkat/L	U/L	µkat/L	U/L	µkat/L	U/L	µkat/L
n		80		80		80		80		80	
Mean		69	1.15	289	4.82	800	13.3	53	0.884	155	2.58
Within Run	SD	1.5	0.025	2.6	0.043	9.9	0.165	1.7	0.028	1.7	0.028
	CV %	2.2		0.9		1.2		3.2		1.1	
Total	SD	1.5	0.025	3.8	0.063	9.9	0.165	1.9	0.032	3.2	0.053
	CV %	2.3		1.3		1.2		3.5		2.1	

CX		Serum						Urine			
		Level 1		Level 2		Level 3		Level 1		Level 2	
		U/L	µkat/L	U/L	µkat/L	U/L	µkat/L	U/L	µkat/L	U/L	µkat/L
n		80		80		80		80		80	
Mean		70	1.17	285	4.75	810	13.5	54	0.900	157	2.62
Within Run	SD	1.8	0.030	3.3	0.055	4.8	0.080	2.5	0.042	2.1	0.035
	CV %	2.6		1.2		0.6		4.6		1.4	
Total	SD	2.3	0.038	4.1	0.068	7.8	0.130	2.8	0.047	2.9	0.048
	CV %	3.3		1.4		1.0		5.2		1.9	

METHOD COMPARISON

Comparison studies were carried out using the NCCLS (CLSI) EP9-A2 guideline.[7] A commercially available Amylase EPS-G7 reagent was used as the reference method (X), using recommended applications on a Roche Hitachi 911® analyzer. The test method (Y) was run with recommended applications on the Beckman Coulter Synchron LX/CX and UniCel DxC analyzers. Serum / plasma and urine samples were assayed in parallel by both the test and reference methods and the results compared by Deming regression. The following statistics were obtained:

LX/DxC	Serum/Plasma		Serum		Plasma		Urine	
	U/L	µkat/L	U/L	µkat/L	U/L	µkat/L	U/L	µkat/L
n	111		70		41		106	
Range	45 - 1864	0.750 - 31.1	45 - 1864	0.750 - 31.1	46 - 1493	0.768 - 24.9	17 - 1884	0.283 - 31.4
X-mean	410	6.83	400	6.68	428	7.15	400	6.67
Y-mean	396	6.60	386	6.45	414	6.91	403	6.72
Slope	0.975		0.973		0.979		1.027	
Intercept	-3.7	-0.062	-3.0	-0.050	-4.8	0.080	-7.6	0.127
r	0.9994		0.9994		0.9995		0.9992	

CX	Serum/Plasma		Serum		Plasma		Urine	
	U/L	µkat/L	U/L	µkat/L	U/L	µkat/L	U/L	µkat/L
n	111		70		41		99	
Range	45 - 1864	0.750 - 31.1	45 - 1864	0.750 - 31.1	46 - 1493	0.768 - 24.9	17 - 1493	0.283 - 24.9
X-mean	410	6.83	400	6.68	428	7.15	332	5.53
Y-mean	395	6.58	386	6.45	411	6.86	350	5.83
Slope	0.972		0.970		0.975		1.065	
Intercept	-3.2	-0.053	-1.5.	-0.025	-6.1	-0.102	-4.1	-0.068
r	0.9991		0.99993		0.9987		0.9975	

Comparison studies were also carried out in the same manner described above, using the BCI Amylase reagent as the reference method (X). The following statistics were obtained:

LX/DxC	Serum/Plasma		Urine	
	U/L	µkat/L	U/L	µkat/L
n	110		101	
Range	29 - 797	0.483 - 13.3	27 - 779	0.450 - 13.0
X-mean	191	3.18	292	4.87
Y-mean	152	2.53	227	3.78
Slope	0.791		0.726	
Intercept	0.5	0.008	15.1	0.252
r	0.9987		0.9983	

CX	Serum/Plasma		Urine	
	U/L	µkat/L	U/L	µkat/L
n	112		110	
Range	29 - 798	0.483 - 13.3	31 - 773	0.517 - 12.9
X-mean	219	3.65	289	4.82
Y-mean	181	3.02	234	3.90
Slope	0.830		0.775	
Intercept	-0.6	-0.010	10.0	0.167
r	0.9975		0.9963	

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System Parameters

INSTRUMENT PARAMETERS:	Synchron CX	Synchron LX/UniCel DxC
Test Name:	AMYX	AMYX
Reaction Type:	Rate 1	Rate 1
Units:	U/L	U/L
Decimal Precision:	X	X
Reaction Direction:	POSITIVE	POSITIVE
Math Model:	LINEAR	LINEAR
Calculation Factor:	6220	6220
Cal. Time Limit:	0	0
Number of Calibrators:	0	0
1		
2		
3		
4		
5		
6		
Primary Wavelength:	410	410
Secondary Wavelength:	560	560
Sample Volume:	7 µL	7 µL
REAGENTS:		
Primary Inject (first) / First Inject		
Compartment/Component:	A	A
Volume / Dispense Volume:	175 µL	175 µL
Add Time / Inject Time:	0 sec	0 sec
Primary Inject (first) / Second Inject		
Compartment/Component:	B	B
Volume / Dispense Volume:	35 µL	35 µL
Add Time / Inject Time:	0 sec	-180 sec
Secondary Inject / Third Inject		
Compartment/Component:	None	None
Volume / Dispense Volume:	0	0
Add Time / Inject Time:	N/A	N/A
REAGENTS:		
Blank		
Start Read:	220 sec	-80 sec
End Read:	280 sec	-20 sec
Reaction 1		
Start Read:	180 sec	180 sec
End Read:	270 sec	300 sec
Reaction 2		
Start Read:	N/A	N/A
End Read:	N/A	N/A

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System Parameters

INSTRUMENT PARAMETERS:	Synchron CX	Synchron LX/UniCel DxC
USABLE RESULT RANGE:		
Lower Limit:	4	4
Upper limit:	1800	2000
ERROR DETECTION LIMITS:		
Reagent Blank/Blank		
ABS Low Limit:	-0.100	-0.100
ABS High Limit:	0.250	0.250
Rate Low limit:	N/A	-1.500
Rate High limit:	N/A	2.200
Mean Deviation:	N/A	2.200
Reaction/Reaction 1		
ABS Low Limit:	-0.100	-0.100
ABS High Limit:	1.500	2.200
Rate Low Limit:	N/A	-1.500
Rate High Limit:	N/A	2.200
Mean Deviation:	N/A	2.200
Reaction 2		
ABS Low Limit:	N/A	-1.500
ABS High Limit:	N/A	2.200
Rate Low Limit:	N/A	-1.500
Rate High Limit:	N/A	2.200
Mean Deviation:	N/A	2.200
SUBSTRATE DEPLETION:		
Initial Rate:	99.999	99.999
Delta ABS:	1.500	2.200
MULTI POINT SPAN:	N/A	N/A

REFERENCES

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3. IFCC primary reference procedures for the measurement of catalytre activity concentrations of enzymes at 37°C. Part 8. Reference procedure for the measurement of catalytre concentration of α-Amylase. Clin Chem Lab Med 2006: 1146-55
4. Shephard MDS, Mazzachi RD. The Clin Biochem 1983; 4: 61-7.
5. Junge, W.et, al. 'Development of assays for the determination of total and pancreatic amylase at 37°C according to the principle recommended by the IFCC.' Clin Biochem. 2001; 34:607 - 15.
6. Tholen, D. W. et. al. 'EP5-A2. Evaluation of precision performance of quantitative measurement methods; Approved guideline – second edition. National Committee for Clinical Laboratory Standards. 2004; Volume 24: Number 25.
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SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		
	Consult instructions for use		Manufactured by
REAG A	Reagent A	REAG B	Reagent B
			Not ReUse

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