LIPASE

OSR6130	**4 x 10 mL**	**R1 Buffer**
	4 x	**R1 Lyo**
	4 x 3.3 mL	**R2**
	2 x	**Calibrator**
OSR6230	**4 x 30 mL**	**R1 Buffer**
	4 x	**R1 Lyo**
	4 x 10 mL	**R2**
	2 x	**Calibrator**

Intended Use
System reagent for the quantitative determination of Lipase activity in human serum on Beckman Coulter AU analyzers.

Summary
Measurements of serum lipase are used in the diagnosis and treatment of acute pancreatitis or pancreatic injury.

Methodology
The lipase procedure is based on the colorimetric method of Imamura, et al.[1] Pancreatic lipase hydrolyzes esters of long chain fatty acids from their triglycerides. The enzyme activity requires the presence of co-lipase. 1,2-Diglyceride is hydrolyzed to 2-monoglyceride and fatty acid. The 2-monoglyceride is then measured by coupled enzyme reactions catalyzed by monoglyceride lipase (MGLP), glycerol kinase (GK), glycerol phosphate oxidase (GPO) and peroxidase (POD).

1,2-Diglyceride $\xrightarrow{\text{Pancreatic Lipase}}$ 2-Monoglyceride + Fatty Acid

2-Monoglyceride $\xrightarrow{\text{MGLP}}$ Glycerol + Fatty Acid

Glycerol + ATP $\xrightarrow{\text{GK}}$ Glycerol-3-Phosphate + ADP

Glycerol-3-Phosphate + O_2 $\xrightarrow{\text{GPO}}$ DAP + H_2O_2

H_2O_2 + 4-AAP + TOOS $\xrightarrow{\text{POD}}$ Quinone Diimine Dye + 4 H_2O

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

Buffers MES/BES (pH 6.8)	27	mmol/L
GPO (Streptococcus sp.)	> 15	KU/L
1,2-Diglyceride Substrate (Egg)	0.828	mmol/L
POD (Horseradish)	> 500	U/L
Monoglyceride Lipase (Bacillus sp.)	> 400	U/L
Colipase (Porcine)	> 15	KU/L
ATP	> 0.85	mmol/L
Glycerol Kinase (S. cannus)	> 100	U/L
TOOS	1.0	mol/L
4-aminophenazone	0.25	mmol/L
Deoxycholate	> 7.3	mmol/L
Cholic acid	> 2.0	mmol/L
TAPS (pH 8.7)	50	mmol/L

Also contains preservatives and surfactants.

Precautions

1. Warning! Irritant. Do not inhale. Avoid contact with skin and eyes.
2. Dangerous for the environment. Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
3. For *in vitro* diagnostic use.
4. Do not ingest. Harmful if swallowed.
5. Contains sodium azide as a preservative which may react with lead joints in copper plumbing to form explosive compounds. Even though the reagent contains minute quantities of sodium azide, drains should be well flushed with water when discarding the reagent.
6. WARNING: POTENTIAL BIOHAZARDOUS MATERIAL.
   The calibrator reagent is manufactured from human serum. Each human serum donor used in the preparation of this material was tested by an FDA approved method for the presence of the antibody to HIV-1/2 and HCV as well as for hepatitis B surface antigen and was not repeatedly reactive. Because no test method can offer complete assurance that HIV-1/2, HCV, hepatitis B virus or other infectious agents are absent from biological materials, all reagents should be handled at the Biosafety Level 2 as recommended for any infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual, *Biosafety in Microbiological and Biomedical Laboratories,* 1993.

Lipase

Preparation of Reagents
1. R1 Working Reagent: Slowly add the contents of R1 buffer to the bottle containing R1 lyophilisate. Mix until lyophilisate is completely dissolved. Return the working solution to the R1 buffer bottle.
2. R2: Ready for use. No preparation is required.
3. Lipase Calibrator: Add 3.0 mL of deionized water to the lipase calibrator, mix until completely dissolved.

Storage and Stability
1. The reagents are stable, if unopened, up to the stated expiration date when stored at 2 - 8°C.
2. Opened reagents are stable for 21 days when stored in the refrigerated compartment of the analyzer.
3. Reconstituted Lipase calibrator is stable for 60 days when stored at 2 - 8°C if uncontaminated.

Indications of Deterioration
Visible signs of microbial growth, turbidity or precipitate, or any change in reagent color may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation
Serum samples, free from hemolysis, are the recommended specimens.
Heparinized plasma can also be used.

Sample Storage and Stability
Lipase activity is stable for several weeks when stored 2 - 8°C or indefinitely frozen (≤ -20°C).

Interfering Substances
Results of studies[2] show that the following substances interfere with Lipase determinations.

The criteria for no significant interference is recovery within 10% of the initial value.

Ascorbate:	No significant interference up to 20 mg/dL Ascorbate
Bilirubin:	No significant Interference up to 12 mg/dL Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 500 mg/dL Intralipid*

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

Significant interference from exogenous lipases found in Triglyceride reagents has been identified, due to carryover in random access analyzers. Set up run to exclude running Triglyceride assays just prior to a lipase assay, or utilize manufacturer's recommended contamination parameters.
The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies.
For further information on interfering substances, refer to Young[3] for a compilation of reported interferences with this test.

In very rare cases gammopathy, especially monoclonal IgM (Waldeström's macroglobulinemia), may cause unreliable results.

Procedure
A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
Lipase Reagent
Lipase Calibrator

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
The frequency of calibration is every 7 days. Calibration is accomplished by use of the Lipase calibrator.

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day.
In addition, controls should be performed after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
Automatically printed out for each sample in U/L. For SI units (µkat/L) the results must be multiplied by 0.0167.

Dynamic Range
The lipase procedure is linear from 3 – 600 U/L. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Expected Values
Adults:[5] 11 – 82 U/L.
Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the Lipase Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[6]

Estimates of precision, based on CLSI recommendations,[4] are consistent with typical performance. The within run precision is less than 5% CV and total precision is less than 10% CV. Assays of control sera were carried out and data reduced following CLSI guidelines above.

N = 100	Within run		Total	
Mean, U/L	**SD**	**CV%**	**SD**	**CV%**
20.2	0.6	3.0	0.9	4.5
99.2	0.7	0.7	3.3	3.3

Method Comparison[6]

Patient samples were used to compare this Lipase Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	AU600
Slope	0.995
Intercept	0.7
Correlation Coeff. (r)	0.9998
No. of Samples (n)	169
Range (U/L)	4 – 488

Sensitivity

Typical change in absorbance for 1 U/L of lipase is 0.17 mAbsorbance.

References

1. Imamura, S., Hirayama, T., Arai, T., Takao, K., Misaki, H.: An enzymatic method using 1,2-diglyceride for pancreatic lipase test in serum: Clin. Chem. 35: 1126, 1989.
2. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-P, 1986.
3. Young, D.S., Effects of drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
4. CLSI/NCCLS, Evaluation Protocol EP-5-T2, 1992.
5. Beckman Coulter Inc. data on samples collected from 200 blood donors in North Texas.
6. Data is on file for specific AU analyzers.

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