MEDICA

IVD CE

REF 10227-4 4 x 20 mL / 10 mL

LIPASE (LIP)

Wedges each contain usable volumes of 20 mL of R1 reagent and 10 mL of R2 reagent.

INTENDED USE

The EasyRA LIP reagent is intended for the quantitative determination of lipase in human serum or plasma (with lithium heparin as anticoagulant), using the MEDICA EasyRA® Clinical Chemistry Analyzer.
For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

Lipase is defined as that group of enzymes which hydrolyze the glycerol esters of long-chain fatty acids. The measurement of lipase activity in serum and other fluids is used to evaluate conditions associated with the pancreas.[1]

PRINCIPLE OF THE PROCEDURE

The chromogenic lipase substrate 1, 2-o-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester is cleaved by the catalytic action of lipase to form 1, 2-o-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6-methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. The lipase activity in the specimen is proportional to the production of methylresorufin in the reaction and can be determined spectrophotometrically.[1]

1, 2-o-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin)-ester $\xrightarrow{\text{Lipase}}$ 1, 2-o-dilauryl-rac-glycerol + glutaric acid-(6-methylresorufin)-ester

1, 2-o-dilauryl-rac-glycerol + glutaric acid-(6-methylresorufin)-ester $\xrightarrow[\text{Decomposition}]{\text{Spontaneous}}$ glutaric acid + methylresorufin

REAGENTS

Lipase Reagent (R1): Buffer

TAPS [(a)]	100 mmol/L
Sodium hydroxide	40 mmol/L
Sodium deoxycholate	34 mmol/L
Sodium azide	7.7 mmol/L

Lipase Reagent (R2): Substrate

(+)-Tartaric acid	9.5 mmol/L
Sodium hydroxide	19 mmol/L
Colipase	460 IU/mL
2-Propanol	0.65 mol/L
DGGMR [(b)]	0.4 mmol/L

Acronyms: (a) = N-Tris(hydroxymethyl)methyl-3-aminoprpanesulfonic scid
(b) = 1, 2-o-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C. Do not use the reagent if it is turbid or cloudy.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Clear unhemolyzed serum or plasma should be used. Lithium heparin coated tubes may be used for plasma collection. Lipase is stable for 5 days at 2 – 8°C or 24 hours at 20 – 25°C.

PROCEDURE

Materials Provided

Medica Lipase Reagent Wedge, REF 10227

Additional materials required

Medica EasyCal Chemistry, REF 10651
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661
Medica Wash1 Wedge, REF 10680*

*The Wash1 wedge is required due to interferences between lipase and other assays on the EasyRA analyzer. When necessary, the EasyRA analyzer will automatically run the wash cycle.

Instructions for Use

The reagent is ready to use as supplied. Place the reagent in the EasyRA analyzer reagent tray located in the reagent area. Only remove the caps when necessary to run a worklist. Keep the reagent tightly capped when not in use. When used this way, the reagent is stable on-board in the refrigerated reagent area of the EasyRA Analyzer for the number of days programmed on the RFID chip on the reagent wedge (60 days maximum).

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration

Medica EasyCal Chemistry, REF 10651, is recommended for the calibration of the assay. The calibration interval (14 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control

It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay daily whenever patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials.

Results

After completion of the assay, the EasyRA Analyzer calculates the LIP concentration from the ratio of the change in the corrected unknown sample's absorbance per minute to the change in the corrected absorbance of the calibrator per minute multiplied by the calibrator value.

$$\text{Lipase (U/L)} = \frac{(\Delta A/Min_{U} - \Delta A/Min_{Blk})_{550} - (\Delta A/Min_{U} - \Delta A/Min_{Blk})_{700}}{(\Delta A/Min_{C} - \Delta A/Min_{Blk})_{550} - (\Delta A/Min_{C} - \Delta A/Min_{Blk})_{700}} \times \text{Cal Value}$$

Where $\Delta A/Min_U$ and $\Delta A/Min_C$ are the change in absorbance values per minute of the unknown and the calibrator respectively, $\Delta A/Min_{Blk}$ is the change in absorbance of the reagent blank, and "Cal Value" is the concentration of lipase in the calibrator (U/L).

Expected Values[2]

Adults: 10 – 150 U/L

These values are guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)

Only unhemolyzed serum or plasma samples should be used.

The EasyRA Analyzer flags any result above 500 U/L as Linearity High "LH." If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the LIP test to 1000 U/L.

PERFORMANCE CHARACTERISTICS[3]

Reportable Range

The reportable range is 6 to 500 U/L. Extended range is 6 to 1000 U/L when half of the sample is used (1:1 dilution).

Inaccuracy/ Correlation (CLSI, EP9-A2)

The following table lists the serum data obtained in a comparison of the Medica Reagent for LIP (y) on the EasyRA Analyzer to the performance of a similar LIP reagent (x) on Hitachi® 911 Analyzer*. The data shown below represents single determinations obtained on the EasyRA Analyzer vs. the average of two replicate serum values obtained on the Hitachi 911 Analyzer.

Number of samples	69	Range of Samples	18 to 478 U/L
Slope	1.0109	y Intercept	-0.0678
Correlation Coefficient	0.9997	Regression Equation:	1.0109*X – 0.0678

The data shown below represents single determinations for plasma obtained on the EasyRA Analyzer (y) vs. the average of two replicate serum values obtained on the EasyRA Analyzer (x).

Number of samples	54	Range of Samples	16 to 488 U/L
Slope	0.9874	y Intercept	1.0049
Correlation Coefficient	0.9996	Regression Equation:	0.9874*X + 1.0049

** Hitachi is a registered trademark of Kabushiki Kaisha Hitachi Seisakusho DBA Hitach, Ltd. CORPORATION JAPAN 6-6, Marunouchi 1-chome, Chiyoda-ku Tokyo JAPAN 100-8220*

Imprecision (CLSI, EP5-A2)

Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level U/L	Within Run SD U/L	Within Run CV %
275.8	3.0	1.1
81.5	1.2	1.4
39.5	1.1	2.8

Total Imprecision:

QC Level U/L	Total Imprecision SD U/L	Total Imprecision CV %
275.8	6.7	2.4
81.5	2.7	3.3
39.5	1.5	3.8

Linearity (CLSI, EP6-A)

Linear from 6 to 500 U/L, based on the linear regression Y = 0.9926*X + 1.3280.

Limit of Blank (LOB):	1.5 U/L	(CLSI, EP17-A)
Limit of Detection (LOD):	3.7 U/L	(CLSI, EP17-A)
Limit of Quantitation (LOQ):	5.6 U/L	(CLSI, EP17-A)

Interfering Substances (CLSI, EP7-A)

Less than 10% interference was classified as "no significant interference."
No significant interference was found with levels up to 100 mg/dL of hemoglobin.
No significant interference was found with levels up to 32 mg/dL of total bilirubin.
No significant interference was found with levels up to 32 mg/dL of direct bilirubin.
No significant interference was found with levels up to 1517 mg/dL of triglycerides (*using Intralipid***).

** Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests. [4,5]

REFERENCES

1 Tietz, N.W., *Fundamentals of Clinical Chemistry,* Saunders Elsevier, St. Louis. p.333 (2008).
2 Tietz, N.W., *Textbook in Clinical Chemistry,* W.B. Saunders, Philadelphia, p 735 (1986).
3 Data on file at Medica.
4 Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.
5 Young, DS., Pestaner, L.C., Gibberman, V.; Effects of drugs on clinical laboratory tests. Clin Chem 21: 246D, 1975.

EasyRA Assay Parameters (LIP)

Primary Wavelength (nm)	550
Secondary Wavelength (nm)	700
Reaction Type	Kinetic
Reaction Direction	Increase
Reagent Blank	Yes
Sample Blank	No
Max. first interval Abs. change	N/A
Reaction Time	9.4 min
Calibration interval (maximum)	14 days
Reagent on-board stability	60 days

Serum/Plasma

Sample volume (µl)	3.0
Diluent volume (µl)	40
Reagent volume R1 (µl)	200
Reagent volume R2 (µl)	100
Decimal Places (default)	1
Units (default values)	U/L
Dilution Factor	1:1 (to extend measuring range)
Linearity	6 to 500 U/L

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