

QuickGel® Touch LD Isoenzyme Procedure

Cat. No. 3338

The QuickGel Touch LD Procedure is intended for the qualitative and quantitative analysis of the lactate dehydrogenase isoenzymes using agarose gel electrophoresis on the SPIFE Touch system.

SUMMARY

Lactate dehydrogenase (LD, EC 1.1.1.27) is an enzyme found in virtually all human tissues with the liver, skeletal muscle, heart and kidney having the greatest concentrations. The wide distribution of LD in body tissues limits the usefulness of total LD determinations in diagnoses. Definitive testing for the source of elevated LD activity may be accomplished with isoenzyme assessment¹.

Five isoenzymes of LD can be demonstrated in human serum. Each isoenzyme is designated by a number which is related to its electrophoretic mobility. The most anodic fraction is designated LD₁ and is found primarily in heart muscle. The most cathodic is LD₅, found primarily in liver and skeletal muscle. The others - LD₂, LD₃ and LD₄ - are found in varying degrees along with LD₁ and LD₅ in all tissues. Since LD₂ is found in highest concentration in normal human serum, the ratio LD₁/LD₂ is therefore less than one. Approximately 12-24 hours following myocardial infarction (MI), there is substantial elevation in LD₁ so that the LD₁/LD₂ ratio following MI is generally greater than 1, a phenomenon referred to as "flipped LD". Peak activity is usually reached on day 3-4 and activity may remain elevated for as long as two weeks after infarction⁴.

The LD "flip" can also be present in pernicious, hemolytic, acute sickle cell or megaloblastic anemias; renal necrosis or in cases of in-vitro or in-vivo hemolysis of any cause⁵.

An elevation of LD₅ can be seen in skeletal (muscle) injuries and degenerative diseases. It is also increased in many types of liver injuries such as cirrhosis, all types of hepatitis, and passive liver congestion⁶.

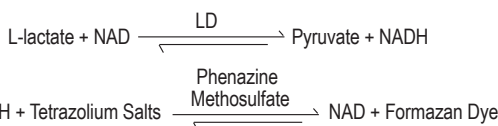
The mid-zone fractions (LD₂, LD₃, LD₄) may be elevated in cases of massive platelet destruction (pulmonary embolism) and in diseases involving the lymphatic system such as infectious mononucleosis, lymphomas and lymphocytic leukemias⁵.

The isoenzymes of LD have been determined by various methods⁷⁻¹¹. Electrophoresis provides far more information than the other methods because it allows complete separation of all five isoenzymes with no risk of carryover. The support media used in electrophoresis includes cellulose acetate, agar, agarose and acrylamide gels¹. The QuickGel LD Isoenzyme system is a modification of that of Preston⁸.

PRINCIPLE

The isoenzymes of LD are separated according to their electrophoretic mobility on agarose. After separation, each isoenzyme is detected colorimetrically.

Using the QuickGel LD, a tetrazolium salt is reduced with the formation of a colored formazan dye.



REAGENTS

1. QuickGel LD Gel

Ingredients: Each gel contains agarose in a sodium barbital buffer, AMPD, aspartic acid, bicine and stabilizers. Sodium azide has been added as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. The gel contains barbital which, in sufficient quantity, can be toxic. Refer to Sodium Azide Warning.

Preparation for Use: The gels are ready for use as packaged.

Storage and Stability: The gels should be stored at room temperature (15 to 30°C), in the protective packaging and are stable until the expiration date indicated on the package. **DO NOT REFRIGERATE OR FREEZE THE GELS.**

Signs of Deterioration: Any of the following conditions may indicate deterioration of the plate: (1) crystalline appearance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial growth indicating contamination, (4) thinning of the gel blocks.

2. QuickGel LD Isoenzyme Reagent

Ingredients (after reconstitution):

NAD	10.0 mM
Lithium Lactate	300.0 mM
NBT	11.1 mM
PMS	0.375 mM

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.

Preparation for Use: Reconstitute each vial of reagent with 1.0 mL of SPIFE LD Diluent.

Storage and Stability: The dry reagent should be stored at 2 to 8°C and is stable until the expiration date on the vial and box. The reconstituted reagent is stable 48 hours at 2 to 8°C when stored in the dark. If exposed to the light, the color will change from yellow to green to blue. This does not affect the performance characteristics of the reagent.

Signs of Deterioration: If the unreconstituted reagent is not a uniformly pale or light yellow, dry powder, it should not be used.

3. LD Diluent

Ingredients: The diluent is an AMP, bicine, barbital, aspartate buffer with sodium azide added as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. Refer to Sodium Azide Warning.

Preparation for Use: The diluent is ready for use as packaged.

Storage and Stability: The diluent should be stored at 2 to 8°C, and is stable until the expiration date on the bottle.

4. Citric Acid Destain

Ingredients: After dissolution, the destain contains 0.3% (w/v) citric acid.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST - IRRITANT.

Preparation for Use: Pour 11 L of deionized water into the Destain vat. Add the entire package of Destain. Mix well until completely dissolved.

Storage and Stability: Store the Destain at 15 to 30°C. It is stable until the expiration date on the package.

Signs of Deterioration: Discard if solution becomes cloudy.

Sodium Azide Warning

To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions. When discarding reagents containing sodium azide, always flush sink with copious quantities of water. This will prevent the formation of metallic azides which, when highly concentrated in metal plumbing, are potentially explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.

INSTRUMENTS

A SPIFE Touch must be used to electrophorese the gels. The gels may be scanned on a densitometer such as the QuickScan Touch/2000 (Cat. No. 1690/1660). Refer to the appropriate Operator's Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Serum is the specimen of choice. Plasma from blood specimens collected in heparin or EDTA may be used. Anticoagulants containing oxalate should not be used due to the inhibition of LD by oxalate¹¹. Plasma samples should be well centrifuged to eliminate platelets which contain LD¹².

Interfering Substances:

1. Hemolysis: Erythrocytes contain 100 to 150 times more LD than does serum. Hemolysis may contribute to error in assessment of LD_{1,2} activity^{1-2,11}.
2. Uremic sera: LD activity is reduced in uremic sera due to the presence of the inhibitors, urea and oxalate, and other unidentified substances. Urea affects LD₅ more than LD₁¹³.
3. Acetone and chloroform inactivate all isoenzymes of LD except LD₁¹⁴.
4. For the effect of various drugs on LD activity, refer to Young et al¹⁵.

Storage and Stability: Serum should be tested as soon as possible after collection. Fresh serum is the specimen of choice because different storage conditions have varying effects on the isoenzymes^{11,14,16,17}. No one storage temperature is optimum for all the isoenzymes. When storage is required, serum samples may be stored at 15 to 30°C or at 2 to 8°C for up to 48 hours. Storage at 2 to 8°C permits simultaneous storage of serum for both CK and LD isoenzyme studies¹¹. Do not freeze the sample as LD₅ is very unstable at freezing temperatures¹¹.

PROCEDURE

Materials Provided: The following materials are provided in the QuickGel LD Kit (Cat. No. 3338). Individual items are not available.

- QuickGel LD Gels (10)
- LD Reagent (10 x 1.0 mL)
- LD Diluent (1 x 10 mL)
- QuickGel Blotter C (10)
- Citric Acid Destain (1 pkg)
- Blade Applicator Kit - 20

Materials provided by Helena but not contained in the kit:

Item	Cat. No.
SPIFE Touch	1068
QuickScan Touch	1690
QuickScan 2000	1660
REP Prep	3100
QuickGel Dispo Cup Tray	3353
SPIFE QuickGel Electrodes	1111
SPIFE QuickGel Gel Holder	3358
CK/LD Control	5134
Gel Block Remover	1115

SPIFE Reagent Spreaders	3706
SPIFE Dispo Sample Cups (Deep Well)	3360
Chamber Cover	8JP34012
SPIFE QuickGel Chamber Alignment Guide	86541003
SPIFE Reagent Spreader	3386
Applicator Blade Weights	3387

STEP-BY-STEP METHOD

I. Stainer Preparation

NOTE: If a SPIFE Touch procedure requiring a stain has been run prior to running the LD gels, the stainer unit must be cleaned/washed before washing the gel.

NOTE: If the staining chamber was last used to stain a gel, the SPIFE Touch software has an automatic wash cycle prompted by the initiation of the QuickGel Touch LD Isoenzyme test. To verify the status of the stainer chamber, use the arrows under the **STAINER UNIT** to select the appropriate test, place the empty Gel Holder into the stainer chamber and press **START**. If washing of the staining chamber is necessary, the prompt "Vat must be washed. Remove gel and install gel holder." will appear. Press **RETRY** to begin the stainer wash. The cleaning process will complete automatically in about 7 minutes. To avoid delays after incubation, this wash cycle should be initiated at least 7 minutes prior to the end of the run.

II. Chamber Preparation

1. The SPIFE QuickGel Chamber Alignment Guide must be used to mark the location for gel placement on the chamber floor if not marked previously. It is recommended that the markings be placed directly on the copper floor under the contact sheet.
2. Remove the contact sheet and clean the chamber floor according to instructions in the Operator's Manual.
3. Place the round hole in the guide over the left chamber pin and the obround hole over the right pin.
4. Using an indelible marker, outline the rectangular open area onto the copper floor. Allow marking to dry, and apply another contact sheet.

III. Sample Preparation

1. Remove one Disposable Applicator Blade from the packaging. If testing more than 10 samples, remove two Applicator Blades from the packaging.
2. Place the Applicator Blade into the vertical slot numbered 6 in the Applicator Assembly. If using two Applicator Blades, place them into the vertical slots numbered 6 and 12.
NOTE: The Applicator Blade will only fit into the slots one way; do not try to force the Applicator Blades into the slots.
3. Place an Applicator Weight on top of the Applicator Blade. When placing the weight on the blade, position the weight with the thick side to the right.
4. Slide the Disposable Sample Cups into the appropriately numbered top row of the Cup Tray. If testing more than 10 samples, place cups into both rows.
5. Pipette 75 to 80 μ L of patient sample or control into cups 1 to 5 and 6 to 10. If testing more than 10 samples, pipette sample into cups 11 to 15 and 16 to 20. Cover the tray until ready to use.

IV. Gel Preparation

1. Carefully open one end of the pouch and remove one gel from the protective packaging. Reseal the pouch with tape to prevent drying of the gel. Remove the gel from the plastic mold and discard the mold.
2. Place a QuickGel Blotter C on the gel with the longer edge parallel with gel blocks. Gently blot the entire surface of the gel using slight fingertip pressure on the blotter, and remove the blotter.
3. Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
4. Place the gel over the REP Prep inside the rectangle on the chamber floor. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side.
5. Use lint-free tissue to wipe around the edges of the gel backing to remove excess REP Prep. Make sure the gel remains in place and that no bubbles remain under the gel.
6. Clean the QuickGel Electrodes and Reagent Spreaders with deionized water before and after each use. Wipe with a lint-free tissue.
7. Place a QuickGel Electrode on the outside ledge of each gel block inside the magnetic posts. Improper contact between the electrodes and the gel block can result in skewed patterns. Close the chamber lid.
8. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test and press **SETUP**.

V. Preparation of Reagent

1. Reconstitute one vial of the LD Isoenzyme Reagent with 1.0 mL LD Isoenzyme Diluent.
2. Mix well by inversion.
3. Place the reconstituted vial of reagent in the center hole of the reagent bar, ensuring that the vial is pushed down as far as it can go. Close the chamber lid.

VI. Electrophoresis/Visualization

Using the instructions provided in the appropriate Operator's Manual, set up the parameters as follows for the SPIFE Touch:

	Separator Unit
Load Sample 1	Prompt: None Time: 0:02 Temperature: 20°C Speed: 6
Load Sample 2	Prompt: None Time: 0:02 Temperature: 20°C Speed: 6
Load Sample 3	Prompt: None Time: 0:02 Temperature: 20°C Speed: 6
Load Sample 4	Prompt: None Time: 0:10 Temperature: 20°C Speed: 6
Apply Sample	Prompt: None Time: 1:00 Temperature: 20°C Speed: 6 Location: 1
Electrophoresis	Prompt: None Time: 4:00 Temperature: 12°C Voltage: 550 V mA: 70 mA
Apply Reagent	Prompt: Remove Gel Blocks Temperature: 45°C Cycles: 4
Incubate	Prompt: To Continue Time: 20:00 Temperature: 45°C
End	
	Stainer Unit
Destain	Prompt: None Time: 10:00 Recirculation: Rev Valve: 2 Fill, Drain
Wash	Prompt: None Time: 5:00 Recirculation: Rev Valve: 7 Fill, Drain
Dry	Prompt: None Time: 13:00 Temperature: 70°C
End	

1. Place the Cup Tray with samples on the SPIFE Touch. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. The SPIFE Touch will apply the samples, electrophorese, and beep when finished.
3. Open the lid, remove the QuickGel Electrodes and dispose of blades as biohazardous waste.
4. With the gel still in the chamber, use a Gel Block Remover to completely remove and discard the two gel blocks.
5. Use a lint-free tissue to wipe around the edges of the gel.
6. Place a Reagent Spreader Rod (glass rod) across each end of the gel inside the magnetic posts. Close the chamber lid and press **CONTINUE** button to spread the reagent.
7. After the reagent is spread, the instrument will beep. Open the chamber lid and insert a Chamber Cover in the grooves of the chamber. Close the chamber lid.

VII. Incubation

1. Press the **CONTINUE** button to start the incubation timer.
2. Once incubation is complete, the instrument will beep. Open the chamber lid. Remove the Chamber Cover and gel from the chamber.
3. Remove the SPIFE QuickGel Holder from the stainer chamber. While holding the gel agarose side down, slide one side of the gel backing under one of the metal bars. Bend the gel backing so that the gel is bowed, and slip the other side under the other metal bar. The two small notches in the backing must fit over the small pins to secure the gel to the holder.
4. Place the SPIFE QuickGel Holder with the attached gel facing backwards into the stainer chamber.

- Use the arrows under **STAINER UNIT** to select the appropriate test. Press **START** and choose an operation to proceed. The instrument will destain, wash and dry the gel.
- When the gel has completed the process, the instrument will beep. Carefully remove the SPIFE QuickGel Holder from the stainer because the metal piece on the holder will be hot.

Evaluation of LD Isoenzyme Bands

- Qualitative evaluation: The QuickGel LD Gel may be visually inspected for the presence of the bands.
- Quantitative evaluation: Scan the QuickGel LD Gel, agarose side up, in the QuickScan Touch/2000 on the Acid Violet setting using slit 5.

Stability of End Product

The LD gels should be scanned for quantitative results within two hours after drying. The gel should be protected from light in the interim. Gels may be kept an indefinite period of time as a permanent record.

Calibration

A calibration curve is not necessary because relative intensity of the bands is the only parameter determined.

Quality Control

The CK/LD Isoenzyme Control (Cat. No. 5134) can be used to verify all phases of the procedure and should be used on each plate run. The control should be used as a marker for proper location of the isoenzyme bands and may also be quantitated to verify the accuracy of quantitations. Refer to the package insert provided with the control for assay values. Additional controls may be required for federal, state or local regulations.

REFERENCE VALUES

Reference range studies including fifty (50) men and women were performed by Helena Laboratories. The following results were obtained:

LD ₁	=	17.7 - 31.5
LD ₂	=	28.0 - 35.7
LD ₃	=	20.8 - 26.8
LD ₄	=	6.4 - 12.7
LD ₅	=	4.5 - 16.0
LD ₁ /LD ₂	=	0.5 - 1.0

These values should only serve as guidelines. Each laboratory should establish its own expected value range with this procedure.

RESULTS

Following electrophoresis, five zones of LD activity can be demonstrated. The most anodic zone (LD₁) migrates with a mobility similar to alpha₂ globulin. The most cathodic zone (LD₅) travels with the gamma globulin and the remaining three zones have intermediate mobilities. The LD activity in normal serum reflects the break-down of numerous cells and all 5 components can be seen. LD₂ predominates, followed by LD₁ and LD₃. LD₄ and LD₅ occur only in minor amounts.

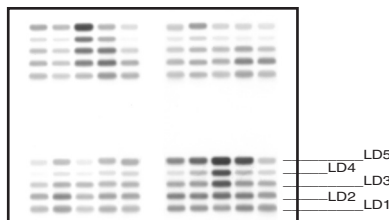


Figure 1: QuickGel LD Gel showing the relative position of the LD Isoenzyme bands.

Calculation of the Unknown

The Helena QuickScan Touch/2000 will automatically calculate and print the relative percent and the absolute values for each band. Refer to the Operator's Manual provided with the densitometer.

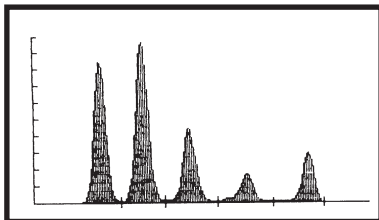


Figure 2: A representative scan of a LD isoenzyme pattern.

LIMITATIONS

The QuickGel LD Isoenzyme Reagent, when used on the SPIFE, is linear to a total LD of 1000 U/L. When used on the QuickGel Chamber, the linearity is to at least 500 U/L. Samples with values greater than this should be diluted with deionized water. Results from sensitivity studies showed that the QuickGel LD Reagent is sensitive to 3 U/L.

NOTE: The QuickGel LD method is not designed to identify tumor markers

Interfering Factors:

Refer to **SPECIMEN COLLECTION AND HANDLING**.

Further Testing Required:

- Total LD activity may be determined. Conflicting reports exist about the true value of total serum enzyme levels as compared to the severity of a disease^{1,4,22}.
- In diagnosing myocardial infarction, CK isoenzyme studies should be performed^{1,4}.
- Haptoglobin studies should be performed to rule out hemolysis as a cause of elevated LD₁ and LD₂.

INTERPRETATION OF RESULTS

- LD₂ is the LD isoenzyme present in the largest amount in normal serum^{1,4,11}.
- LD₁ is elevated and may be greater than LD₂ in:
 - Myocardial infarction^{1,4,11}
 - Duchenne's muscular dystrophy presents a pattern like MI but clinical symptoms help in easily differentiating the two diseases¹⁸⁻¹⁹.
 - Hemolysis (including Hemolytic anemias) should be strongly considered whenever total serum LD reaches levels greater than 5 times normal and the isoenzymes show an increased LD₁ and LD₂. Total LD is much higher in hemolytic anemia than in MI unless MI is accompanied by severe shock. Pernicious anemia (PA) in relapse gives an LD pattern like hemolysis. Some of the highest total serum LD values are found in PA^{2,14}.
 - Renal infarct^{2,11}
- LD₃ is elevated in pulmonary infarctions^{6,11,20}.
- LD₄ elevation has not been associated with any particular pathology.
- LD₅ is elevated in hepatic and muscular damage and diseases of the skin¹.
- Isomorph patterns

When total LD is markedly elevated but all the isoenzymes are of normal percentages, the phenomenon is referred to as an isomorph pattern. Widely divergent groups of clinical diagnoses have shown this type of pattern and include cardiorespiratory diseases, malignancy, fracture, diseases of the central nervous system, infection/inflammation, hepatic cirrhosis and/or alcoholism, trauma without fracture, infectious mononucleosis, hypothyroidism, uremia, necrosis, pseudomononucleosis, viremia and intestinal obstruction. (See LIMITATIONS Note)

- CK and LD values following open heart surgery: CK and LD isoenzymes are less specific following open heart surgery than they are in most diagnostic situations. The CK-MB will be elevated due to myocardial damage resulting from the operative procedure as well as trauma to the heart from manipulation and cannulation. The LD₁/LD₂ ratio may be elevated secondary to hemolysis from extra corporeal circulation.

PERFORMANCE CHARACTERISTICS

PRECISION

Within Run: A patient sample was run in replicate on a single gel with the following results:

Patient (N=6)

Fraction	Mean	SD	CV
LD ₁	21.3	0.6	2.7%
LD ₂	30.5	0.6	1.1%
LD ₃	22.6	0.5	2.0%
LD ₄	10.4	0.3	2.6%
LD ₅	15.2	0.5	3.1%

Between Run: A patient sample was run in replicate on nine gels with the following results:

Patient (N=90)

Fraction	Mean	SD	CV
LD ₁	21.6	0.9	4.2%
LD ₂	30.9	1.1	3.4%
LD ₃	21.5	1.1	4.9%
LD ₄	10.5	1.6	15.0%
LD ₅	15.4	1.8	11.5%

CORRELATION

Twenty normal and abnormal patient specimens plus a control were analyzed using both the SPIFE Touch and SPIFE 3000. The QuickGel LD method was used as the reference method.

N	=	20
Slope	=	0.9104
Intercept	=	1.7996
R	=	0.9937
Y	=	0.9104X + 1.7996
X	=	QuickGel LD on SPIFE 3000 (Control Plate)
Y	=	QuickGel LD on SPIFE Touch

LINEARITY

QuickGel LD showed linearity up to a total LD of 1000 U/L.

SENSITIVITY

Results from validation studies show that the system is sensitive to 3 U/L.

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QuickGel LD System

QuickGel LD Kit

Cat. No. 3338

QuickGel LD Gels (10)
LD Reagent (10 x 1.0 mL)
LD Diluent (1 x 10 mL)
QuickGel Blotter C (10)
Citric Acid Destain (1 pkg)
Blade Applicator Kit -20

Other Supplies and Equipment

The following items, needed for performance of the QuickGel Touch LD Procedure, must be ordered individually.

	Cat. No.
SPIFE Touch	1068
QuickScan Touch	1690
QuickScan 2000	1660
REP Prep	3100
QuickGel Dispo Cup Tray	3353
SPIFE QuickGel Electrodes	1111
SPIFE QuickGel Gel Holder	3358
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SPIFE Reagent Spreader	3386
Applicator Blade Weights	3387

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