

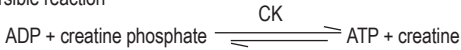
QuickGel® Touch CK Vis Isoenzyme Procedure

Cat. No. 3334

The QuickGel® Touch CK Vis Isoenzyme Procedure is intended for the qualitative and quantitative analysis of the creatine phosphokinase isoenzymes in serum by agarose electrophoresis using the SPIFE Touch system.

SUMMARY

Creatine phosphokinase (CK) (EC 2.7.3.2) is an energy transfer enzyme which catalyzes the reversible reaction



CK exists primarily in skeletal muscle, cardiac muscle and the brain, with small amounts in several other tissues¹. A number of diverse clinical episodes such as surgical procedures, intramuscular injections and myocardial infarct induce increased CK activity in the serum^{2,3}. The source of elevated CK activity may be narrowed by isoenzyme assessment. There are two molecular CK subunits, designated M and B, the combinations of which produce three isoenzymes: CK-MM (isolated primarily from skeletal muscle), CK-MB (myocardium) and CK-BB (primarily from the brain)³.

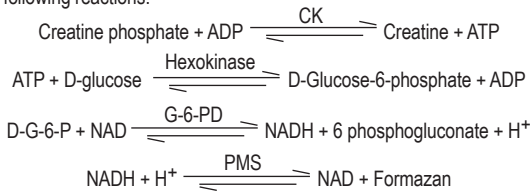
CK isoenzyme analysis is one of the important procedures used in the detection of myocardial damage⁴. After an acute myocardial infarction (MI), CK-MB appears in the serum in approximately 4 to 6 hours, reaches peak activity at 18 to 24 hours, and may disappear completely within 72 hours. Within the first 48 hours after MI, CK-MB is present in 100% of the patients with MI as well as in some cases of severe coronary insufficiency^{1,3,7}.

Definitive laboratory testing in the diagnosis of MI is accomplished by performing studies of CK isoenzymes in conjunction with lactate dehydrogenase (LD) isoenzymes^{3,5-9}. The specificity and sensitivity achieved with these two tests has eliminated the necessity for additional enzyme studies in accurately diagnosing MI⁵. The most important consideration in the interpretation of CK and LD isoenzyme patterns is the detection of the characteristic change of pattern of multiple examinations (the relatively fast appearance and disappearance of CK-MB and the flip of LD₁ and LD₂)^{1,3,35}. Persistent elevation in CK-MB is not indicative of myocardial infarct. CK-MB may be helpful in diagnosing a small infarct in which total CK never exceeds the upper limit of normal⁹.

CK produced by myocardium is only 25 to 40% CK-MB, the remainder being CK-MM^{1,4}. Therefore, an elevation in CK due to myocardial infarction produces not only a rise in CK-MB but in CK-MM as well⁵. The isoenzymes of CK have been assessed by various methods¹⁰⁻¹⁹. Electrophoresis offers the distinct advantage of complete separation of the isoenzymes without risk of carryover³.

PRINCIPLE

The isoenzymes of CK are separated according to their electrophoretic mobility on agarose gel. After separation the gels are incubated with the SPIFE CK Isoenzyme Reagent which utilizes the following reactions:



REAGENTS

1. QuickGel CK Gel

Ingredients: Each gel contains agarose in a AMP/MOPSO buffer. Sodium azide has been added as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE. 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 gel: (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 gel blocks.

2. CK Vis Isoenzyme Reagent

Ingredients:

Adenosine 5'-diphosphate (ADP)	12 mM
Creatine phosphate	90 mM
Adenosine 5'-monophosphate (AMP).....	15 mM
Magnesium Acetate	60 mM
Diadenosine pentaphosphate.....	0.1 mM
Nicotinamide adenine dinucleotide (NAD).....	10 mM
D-glucose.....	60 mM

Glucose-6-phosphate dehydrogenase (L.mesenteroides)	7,500 IU/L
Hexokinase (Yeast).....	9,000 IU/L
PMS	0.15 mM
Bovine Serum Albumin (BSA).....	4.5 g/L

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. Preparation for Use: Reconstitute one vial of CK Reagent with 1.5 mL of CK 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. Reconstituted reagent is stable for 1 hour at 15 to 30°C.

Signs of Deterioration: If the unreconstituted reagent is not a uniformly white or slightly off white dry powder, it should not be used.

3. CK Diluent

Ingredients: The diluent contains MES, sucrose, Triton X and sodium azide added as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE. 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 indicated on the vial.

Signs of Deterioration: Discard the diluent if it shows signs of bacterial growth.

4. CK Chromogen

Ingredients: The chromogen contains 0.023 g Tetranitro Blue Tetrazolium (TNBT) per mL Dimethyl-formamide.

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

Preparation for Use: Add 150 µL of Chromogen to each vial of reconstituted Reagent, invert and use immediately.

Storage and Stability: The Chromogen should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.

Signs of Deterioration: The product should be discarded if it shows noticeable signs of turbidity.

5. CK Activator

Ingredients: The Activator contains 114 mM BME (Beta Mercapto Ethanol) in Tris base.

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

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

Storage and Stability: The Activator should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.

Signs of Deterioration: The product should be discarded if it shows noticeable signs of turbidity.

6. Citric Acid Destain

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

WARNING: FOR IN-VITRO DIAGNOSTIC USE. 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.

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 and dry the gel. 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 operating instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Serum is the specimen of choice.

Collection of Specimen: Proper timing of specimen collections is critical to accurate interpretation of CK isoenzyme analysis. A blood specimen should be obtained immediately upon admission of the patient to the hospital and at 8 to 12 hour intervals thereafter for a minimum of 36 hours.

Interfering Substances:

1. Mature red blood cells contain no CK; however, some of the side reactions may occur in the coupled enzyme assay resulting in lower estimated CK activity. Non-hemolyzed samples are, therefore, preferred²⁰.
2. CK is inactivated by heat²⁰.
3. Repeated freezing and thawing destroys activity (see Serum Storage).
4. For the effects of various drugs on CK activity, refer to Young, et al²¹.

Serum Storage:

1. The blood specimen should be refrigerated (2 to 8°C) immediately after collection. Serum should be separated from the red blood cells as soon as possible.
2. Serum specimens may be stored at 2 to 8°C for up to 48 hours²².
3. Specimens may be stored frozen (-20°C) for up to two weeks²². Frozen specimens should be thawed at room temperatures and should never be placed in a 30 to 37°C water bath for thawing. Repeated freezing and thawing destroys CK activity and should be avoided.

PROCEDURE FOR SPIFE

Materials Provided: The following materials are provided in the QuickGel CK Vis Isoenzyme Kit (Cat. No. 3334). Individual items are not available.

QuickGel CK Gels (10)
CK Vis Reagent (10 x 1.5 mL)
CK Diluent (1 x 15 mL)
CK Chromogen (1 x 1.5 mL)
CK Activator (1 x 0.2 mL)
QuickGel Blotter C (10)
Citric Acid Destain (1 pkg)
Blade Applicator Kit – 20 Sample

Materials provided but not contained in the kit:

Item	Cat. No.
SPIFE Touch	1068
QuickScan Touch	1690
Quick Scan 2000	1660
Disposable Sample Cups (Deep Well)	3360
CK/LD Control	5134
REP Prep	3100
Gel Block Remover	1115
SPIFE Reagent Spreaders	3706
Applicator Blade Weights	3387
SPIFE Reagent Spreader	3386
QuickGel Dispo Cup Tray	3353
SPIFE QuickGel Electrode	1111
SPIFE QuickGel Holder	3358
SPIFE QuickGel Chamber Alignment Guide	86541003
Chamber Cover	8JP34012

STEP-BY-STEP METHOD

I. Stainer Preparation

NOTE: If a SPIFE Touch procedure requiring a stain has been run prior to running the CK gels, the stainer unit must be cleaned/washed before washing the CK 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 CK Vis 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 Quick Gel Chamber Alignment Guide must be used to mark the location for gel placement if the chamber floor hasn't been 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. Add 1 µL Activator to 100 µL patient sample or control. Mix and allow to sit at room temperature for 10 minutes.
2. If testing fewer than 10 samples, remove one Applicator Blade Assembly from the packaging. If testing 11 to 20 samples, remove two disposable Applicator Blade Assemblies from the packaging.
3. Place the Applicator Blade into the vertical slot 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 Blade into the slots.
4. 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.
5. Slide the Disposable Sample Cups into the appropriately numbered top row of the Cup Tray. If testing more than 10 samples, place the cups into both rows.
6. Pipette 75 to 80 µL of pretreated patient serum or control into cups numbered 1 to 5 and 6 to 10. If testing more than 10 samples, pipette samples into cups 11 to 15 and 16 to 20. Cover the tray until ready to use.

IV. Gel Preparation

1. Carefully cut open the end of the gel pouch. Remove one gel from the protective packaging. Fold and tape the open end of the pouch 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. Use lint-free tissue to wipe around the edges of the plastic gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure the gel remains in place and that no bubbles remain under the gel.
5. Clean the QuickGel Electrodes and Reagent Spreaders with deionized water before and after each use. Wipe with a lint-free tissue.
6. 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.
7. Use the arrows under **SEPARATOR UNIT** to select the appropriate test. To check parameters, select test and press **SETUP**.

V. Preparation of Isoenzyme Reagent

1. Reconstitute the CK Reagent with 1.5 mL CK Diluent. Mix well by inversion during the electrophoresis process. Do not add the Chromogen until ready to use as it can cause excess background on the gel.
2. Place a reconstituted vial of reagent (without Chromogen) into the center hole of the reagent bar on SPIFE Touch, 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 Operator's Manual, set up the parameters as follows for the SPIFE Touch:

***Due to variation in environmental conditions, a Dry time of 15 minutes is recommended, but a range of 12 to 20 minutes is acceptable.**

	Separator Unit
Load 1	Prompt: None Time: 0:02 Temperature: 21°C Speed: 6
Load 2	Prompt: None Time: 0:02 Temperature: 21°C Speed: 6
Load 3	Prompt: None Time: 0:02 Temperature: 21°C Speed: 6
Load 4	Prompt: None Time: 0:30 Temperature: 21°C Speed: 6
Apply 1	Prompt: None Time: 1:00 Temperature: 21°C Speed: 6 Location: 1
Load 5	Prompt: None Time: 0:30 Temperature: 21°C Speed: 6
Apply 2	Prompt: None Time: 1:00 Temperature: 21°C Speed: 6 Location: 1
Electrophoresis	Prompt: None Time: 10:00 Temperature: 13°C Voltage: 225 V mA: 30 mA
Apply Reagent	Prompt: Remove Gel Blocks Temperature: 37°C Cycles: 8
Incubate	Prompt: To Continue Time: 18:00 Temperature: 45°C
End	

	Stainer Unit
Destain 1	Prompt: None Time: 2:30 Recirculation: On Valve: 2 Fill, Drain
Destain 2	Prompt: None Time: 2:30 Recirculation: On Valve: 2 Fill, Drain
Wash 1	Prompt: None Time: 2:30 Recirculation: On Valve: 7 Fill, Drain
Wash 2	Prompt: None Time: 2:30 Recirculation: On Valve: 7 Fill, Drain
Dry	Prompt: None Time: 15:00* Temperature: 63°C

End

- Place the Cup Tray with samples on the SPIFE Touch. Align the holes in the tray with the pins on the instrument.
- 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.
- Open the chamber lid, remove the electrodes and dispose of blades as biohazardous waste.
- With the gel still in the chamber, use a Gel Block Remover or straight edge to completely remove and discard the two gel blocks.
- Use a lint-free tissue to wipe around the edges of the gel.
- Place a Reagent Spreader Rod (glass rod) across each end of the gel inside the magnetic posts.
- Remove the reagent vial and add 150 µL of Chromogen to it. Invert to mix, and replace immediately into the center hole of the reagent bar, ensuring that the vial is pushed down as far as it can go. Close the chamber lid.
- Press the **CONTINUE** button to spread the reagent.
- 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

- Press the **CONTINUE** button to start the incubation timer.
- At the end of the incubation, the instrument will beep. Remove the gel from the chamber.
- 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.
- 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 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 the CK Isoenzyme Bands

- Qualitative evaluation: The QuickGel CK Vis Isoenzyme Gel may be visually inspected for the presence of the bands.
- Quantitative evaluation: Scan the QuickGel CK Vis Isoenzyme Gel, agarose side up, in the Quick Scan Touch/2000 using the Acid Violet filter. A slit size of 5 is recommended.

Stability of End Product

The CK gels should be scanned for quantitative results within two hours after drying.

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 gel 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 50 normal men and women were performed by Helena Laboratories. The following results were obtained:

	Range
% MM	97 - 100
% MB	0 - 3
% BB	0

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

RESULTS

CK-BB is the fastest moving, most anodic band, CK-MM is the slowest moving, most cathodic band, and CK-MB migrates intermediate to CK-MM and CK-BB.^{1, 2, 3}

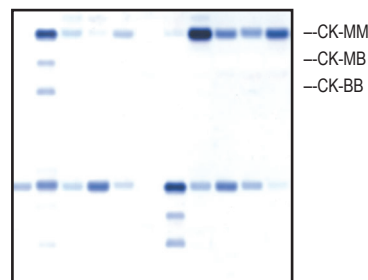


Figure 1: A representation of a QuickGel CK Vis Isoenzyme Gel showing the relative position of the CK Isoenzyme bands.

Calculation of the Unknown

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

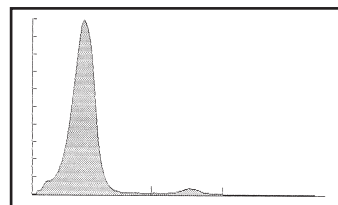


Figure 2: QuickGel CK Vis electrophoresis scan.

LIMITATIONS

The QuickGel CK Vis Isoenzyme Reagent is linear to 700 U/L total CK as determined with a UV kinetic method at 37°C. Results for sensitivity studies show that the CK Reagent is sensitive to 3 U/L.

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

Interfering Factors: Refer to SPECIMEN COLLECTION AND HANDLING.

Further Testing Required: Lactate dehydrogenase (LD) isoenzyme studies performed in conjunction with the CK isoenzymes provide a much more definitive test in the diagnosis of myocardial infarct^{2, 3}.

INTERPRETATION OF RESULTS

CK-MM

- Often the only isoenzyme of CK found in normal serum¹.
- Elevated in: (a) Skeletal muscle injury (b) Myocardial injury (c) Brain injury^{1, 3}.

CK-MB

- May be present in serum from normal subjects in the amount of 0 to 5%²³. Note that small amounts of CK-MB activity have been interpreted as an alert to possible myocardial infarct and should be followed by serial CK and LD isoenzyme studies.
- Positive indication of myocardial infarct when the following criteria are met:
 - Proper clinical setting².
 - CK-MB activity > 5% of total CK activity and a minimum of 10 IU/L^{1, 14, 24}.
 - CK-MB shows characteristic change in pattern (relatively rapid appearance and disappearance)^{1, 3, 35}.
- Positive identification of second myocardial infarct: After the first MI the CK-MB increases after starting to decline. The total CK may or may not show an increase after starting to decline.
- Values following open heart surgery³

CK and LD isoenzymes are less specific following open heart surgery than 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 LD is flipped secondary to hemolysis from extra corporeal circulation. Infarct patients have higher levels of CK-MB activity, but the wide range of isoenzyme activity seen in non-MI patients overlaps that noted in patients with MI. This makes complete discrimination impossible. Despite this difficulty, accuracy in diagnosing MI can be increased by doing serial determinations of CK-MB in the post-operative period and analyzing its activity trend. Perioperative infarct patients will usually have a progressive rise in CK-MB levels, while non-MI patients exhibit a more precipitous post-operative decrease in that fraction^{2, 25}.

5. Elevation in diseases other than myocardial infarct^{1,3}.

Severe coronary insufficiency	Dermatomyositis
Duchenne's muscular dystrophy	Myoglobinuria
Rocky Mountain Spotted Fever	Polymyositis
Rhabdomyolysis	Reye's Syndrome

CK-BB

- Often seen in the serum of patients with prostatic carcinoma and occasionally in the serum of patients with other carcinomas and malignant tumors¹.
- Rarely seen in the serum of patients with brain injury due to damage to the blood-brain barrier^{1,26}.
- Occasionally seen in the serum of patients with severe shock syndrome (probably due to lung or small bowel involvement).
- Occasionally seen in the serum of patients with chronic renal failure, gastric cancer, women in labor, Reye's syndrome, oat cell carcinoma, and malignant hyperpyrexia¹.

ATYPICAL CK BANDS

A number of atypical bands of CK have been reported. Atypical bands migrating between CK-MB and CK-MM have been attributed to CK-BB complexed to IgG^{27,28} and CK-MM complexed to lipoprotein,²⁹ as well as others without positive identification³⁰⁻³². Mitochondrial CK migrates cathodically to CK-MM³³, and a band designated "macro" CK, isolated from a cancer patient, also migrated cathodic to CK-MM³⁴.

PERFORMANCE CHARACTERISTICS

PRECISION

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

	Fraction	Mean	SD	CV%
N = 10	% MM	63.9	0.6	0.9
	% MB	14.2	0.4	2.6
	% BB	22.0	0.3	1.4

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

	Fraction	Mean	SD	CV%
N = 90	% MM	63.6	1.3	2.0
	% MB	14.3	0.6	4.3
	% BB	22.1	0.8	3.5

CORRELATION STUDIES

20 specimens were analyzed using the QuickGel CK method on the SPIFE 3000 and the QuickGel CK method on the SPIFE Touch with the following results:

N	= 20	Y = 0.9974X + 0.2073
Slope	= 1.9974	X = QuickGel CK Vis on SPIFE 3000
Intercept	= 1.2073	Y = QuickGel CK Vis on SPIFE Touch
R	= 1.0	

LINEARITY

The systems have been validated to be linear to 700 U/L total CK.

SENSITIVITY

Results from validation studies show that the systems are sensitive to 3 U/L.

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QuickGel CK Vis Isoenzyme System	
QuickGel CK Vis Isoenzyme Kit	Cat. No. 3334
QuickGel CK Vis Isoenzyme Gels (10)	
CK Vis Isoenzyme Reagent (20 x 1.5 mL)	
CK Vis Isoenzyme Diluent (2 x 15 mL)	
CK Vis Chromogen (2 x 1.5 mL)	
CK Vis Activator (2 x 0.2 mL)	
QuickGel Blotter C (10)	
Citric Acid Destain (1 pkg)	
Blade Applicator Kit-20 Sample	
Other Supplies and Equipment	
The following items, needed for performance of the QuickGel Touch CK Vis Isoenzyme Procedure, must be ordered individually.	
Item	Cat. No.
SPIFE Touch	1068
QuickScan Touch	1690
Quick Scan 2000	1660
Disposable Sample Cups (Deep Well)	3360
CK/LD Control	5134
REP Prep	3100
Gel Block Remover	1115
SPIFE Reagent Spreaders	3706
Applicator Blade Weights	3387
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SPIFE QuickGel Holder	3358
SPIFE QuickGel Chamber Alignment Guide	86541003
Chamber Cover	8JP34012

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