

REP® CK-16 Isoenzyme Procedure

Helena Laboratories

Cat. No. 3071

The REP CK-16 Isoenzyme Procedure is intended for the qualitative and quantitative analysis of the creatine phosphokinase isoenzymes by agarose electrophoresis.

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 tissues.¹ 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 most important procedures used in the early detection of myocardial damage.⁴ After an acute myocardial infarction (MI), CK-MB appears in the serum in approximately 4 to 6 hours, reaches peak activity at 18-24 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} The most definitive laboratory testing in the diagnosis of MI is accomplished by performing studies of CK isoenzyme in conjunction with lactate dehydrogenase (LD) isoenzymes.^{3,5-8} The specificity and sensitivity achieved with these two tests has eliminated the necessity for additional enzyme studies in accurately diagnosing MI.⁶ 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 over 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 normal.⁹

CK produced by myocardium is only 25-40%, 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 well.³ The isoenzymes of CK have been assessed by various methods.¹⁰⁻¹⁹ Electrophoresis offers the distinct advantage of complete separation of the isoenzymes without risk of carryover.³

PRINCIPLE

The isoenzymes of CK are separated according to their electrophoretic mobility on agarose gel. After separation the gels are incubated with the REP CK-16 Isoenzyme Reagent. The REP CK-16 Isoenzyme Reagent (substrate) utilizes the following reactions:



REAGENTS

1. REP CK-16 Isoenzyme Gel

Ingredients: Each gel contains agarose in a Tris Barbitol buffer. Thimerosal has been added as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.

CAUTION: Contains barbitol which, in sufficient quantity, can be toxic.

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.

2. REP CK-16 Isoenzyme Reagent

Ingredients:

Adenosine 5'-diphosphate (ADP)	12 mM
Creatine phosphate	90 mM
Adenosine 5'-monophosphate (AMP)	15 mM
Magnesium Acetate	60 mM
Nicotinamide adenine dinucleotide (NAD)	6 mM
N-Acetylcysteine (NAC)	60 mM
D-glucose	60 mM
Glucose-6-phosphate dehydrogenase	7,500 IU/L
Hexokinase	9,000 IU/L

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

Preparation for Use: Reconstitute each vial of REP CK-16 Isoenzyme Reagent with 1 mL of REP CK Isoenzyme Diluent.

Storage and Stability: The dry reagent should be stored at 2 to 6°C and is stable until the expiration date on the vial. The reconstituted reagent is stable for 4 hours at 15 to 30°C or 48 hours stored at 2 to 6°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. REP CK Isoenzyme Diluent

Ingredients: The diluent contains MES, sucrose and 0.01% sodium azide has been added as a preservative.

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

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.

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

Storage and Stability: The diluent should be stored at 2 to 6°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.

INSTRUMENTS

A Rapid ElectroPhoresis Analyzer (REP or the REP 3) must be

used to electrophorese, apply reagent, incubate, dry and scan the gel. 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, preferred.²⁰
2. Fluorescent activity may be lost due to the quenching effect of water contaminants.
3. CK is inactivated by heat.²⁰
4. Repeated freezing and thawing destroys activity (see Serum Storage).
5. For the effects of various drugs on CK activity, refer to Young, et al.²¹

Serum Storage:

1. The blood specimen should be refrigerated (2 to 6°C) immediately after collection. Serum or plasma should be separated from the red blood cells as soon as possible.
2. Serum specimens may be stored at 2 to 6°C for up to 48 hours.²²
3. Specimens may be stored frozen (-20°C) for up to two weeks.²² Frozen specimens should be thawed at room temperatures and should never be placed in a 30°C or 37°C water bath for thawing. Repeated freezing and thawing destroys CK activity and should be avoided.

PROCEDURE

Materials Provided: The following materials are provided in the REP CK-16 Isoenzyme Kit. Individual items are not available.

- REP CK-16 Isoenzyme Gels (10)
- REP CK-16 Isoenzyme Reagent (10 x 1 mL)
- REP CK Isoenzyme Diluent (1 x 15 mL)
- REP Blotter A (10)
- REP Sample Cups (160 cups)

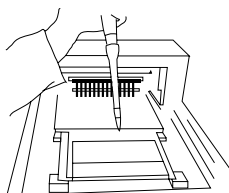
Materials provided by Helena but not contained in the kit:

	Cat. No.
REP CK/LD Isoenzyme Control	3073
REP Prep Solution	3100
SUREprep	1574
REP Prepper	1359

STEP-BY-STEP METHOD

A. Preparation of Isoenzyme Reagent

1. Reconstitute the REP CK-16 Isoenzyme Reagent with 1 mL REP CK Isoenzyme Diluent.
2. Mix well by inversion.

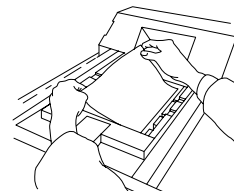


B. Sample Application

1. Place 8 cups into wells 4, 5, 6, 7, 8, 9, 10 and 11; and 8 cups into wells 19, 20, 21, 22, 23, 24, 25 and 26 (color coded with green stripe). Place 50-75 µL of sample in each sample cup. Place REP Blotter A on sample tray in area adjacent to sample cups. Place approximately 4 mL of SUREprep into outside washwell of sample tray. Place approximately 4 mL of water into inside washwell of sample tray.
2. Dispense approximately 1 mL of REP Prep solution onto left side of REP chamber.
3. Remove the gel from the protective packaging and discard overlay. Inspect the scanning area of the gel for

surface artifacts. Use the REP Prepper or compressed air to remove excess moisture from the wells.

4. Place the left edge of the gel over REP chamber, aligning the round hole on the left pin. Gently lay the gel down on the REP Prep starting from the left side and ending on the right side, fitting the obround hole over the right pin. Use paper towel or absorbent paper to wipe around the edges of the gel, especially next to electrode posts to remove excess surfactant. Make sure that the gel lays flat in the chamber and that no bubbles remain under the gel.



5. Clean the electrodes with deionized water and wipe with a lint-free tissue before and after each use.
6. Place a carbon electrode on each gel block inside the magnetic posts.
7. Place the open vial of reconstituted reagent firmly in the center vial holder (color coded with a green stripe).
8. Slide the lid into place until it snaps.
9. Using the instructions provided in the appropriate Operator's Manual, set up parameters on the screen as follows:

REP

Sample Location [Row]	AB
Sample Application Time	2 sec
Sample Application Volume	1 µL
Sample Absorption Time	1:00 mm:ss
Needle Wash Cycles	2
Needle Blot Time	1 sec
Electrophoresis Time	2:30 mm:ss
Electrophoresis Voltage	1200 volts
Electrophoresis Current	0 mA
Electrophoresis Temperature	13°C
Air Dry Time	mm:ss
Reagent Pour Time	1 sec
Reagent Spread Cycles	4
Incubation Time	4:30 mm:ss
Incubation Temperature	45°C
Dry Time	5:00 mm:ss
Dry Temperature	54°C
Standby Temperature	16-20°C

Depress the F1 key, and the REP unit will automatically apply samples, electrophorese, apply reagent, incubate and dry the agarose gel.

REP 3

Sample Application Volume	1.0 µL
Sample Application Row A	66.50 mm from front pin
Sample Application Row B	117.00 mm from front pin
Sample Absorption Time	00:40 min:sec
Electrophoresis Voltage	1200 volts
Electrophoresis Current Limit	300 mA
Electrophoresis Temperature	13°C
Electrophoresis Time	02:30 min:sec
Air Dry Time	00:00 min:sec
Reagent Spread Cycles	4
Reagent Absorption Time	00:02 min:sec
Center Electrode State	None
Incubation Temperature	45°C
Incubation Time	04:30 min:sec
Dry Temperature	54°C
Dry Time	05:00 min:sec

Touch the "Start Run" area on the touch screen. The REP 3 will automatically apply samples, electrophorese, apply reagent, incubate, dry and scan the gel.

C. Evaluation of the CK Isoenzyme Bands

1. Qualitative evaluation: The REP CK-16 Isoenzyme Gel may be visually inspected for the presence of bands. Inspection should be done in a dark environment with a high quality U.V. lamp source. The TITAN UV Lamp (Cat. No. 5031) is recommended.
2. Quantitative evaluation: Scan the REP CK-16 Isoenzyme Gel in the REP using the fluorescence mode. Place the agarose side of the gel down toward the detector. The REP 3 automatically scans the gels.

Stability of End Product

The CK gels should be inspected visually and/or scanned for quantitative results within one hour after drying. The gel should be protected from light in the interim.

Calibration

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

Quality Control

The REP CK/LD Isoenzyme Control (Cat. No. 3073) 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.

RESULTS

CK-BB (CK1) is the fastest moving, most anodic band, CK-MM (CK3) is the slowest moving, most cathodic band, and CK-MB (CK2) migrates intermediate to CK-MM and CK-BB.^{1,2,3}



Figure 1: A representation of a REP CK-16 Isoenzyme Gel showing the relative position of the CK Isoenzyme bands.

Calculation of the Unknown

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

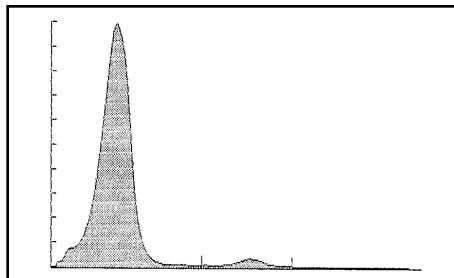


Figure 2: REP CK-16 electrophoresis scan made with a Helena REP densitometer.

LIMITATIONS

The REP CK Isoenzyme Reagent is linear to 1000 IU/L total CK as determined with a UV kinetic method at 37°C. Results for sensitivity studies show that the REP CK Reagent is sensitive to 3 IU.

NOTE: The REP CK-16 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}

REFERENCE VALUES

REP

Normal range studies including both men and women were performed by Helena Laboratories. The following results were obtained:

CK-MM = 97-100% CK-MB = 0-3% CK-BB = 0%

REP 3

Reference range studies were performed by Helena Laboratories on 60 samples from healthy men and women. The results were as follows:

CK-MM = 100% CK-MB = 0% CK-BB = 0%

These values should only serve as guidelines. Each laboratory should establish its own range.

PERFORMANCE CHARACTERISTICS

REP

PRECISION

Within Run studies were done using a control run 16 times on one gel. The results were as follows: N = 16

	%MM	%MB	%BB
\bar{X}	64.5	9.8	25.8
SD	0.7	0.3	0.8
CV%	1.1	3.0	3.0

Between Run studies were done using a control run 16 times on each of 11 gels. The results were as follows: N = 176

	%MM	%MB	%BB
\bar{X}	63.0	9.8	27.1
SD	1.6	0.6	1.5
CV%	2.6	6.2	5.4

CORRELATION

The Helena REP CK-16 method was compared to the REP CK-30 method with the following correlation:

$$N = 13 \quad Y = 1.033X - 1.647$$

$$\text{Slope} = 1.033 \quad X = \text{REP CK-30}$$

$$\text{Intercept} = -1.647 \quad Y = \text{REP CK-16}$$

$$r = 0.999$$

REP 3

PRECISION

Within Run studies were run using a control run in replicate on one gel. N = 16

	% MM	% MB	% BB
Mean	75.4	6.2	18.4
SD	0.6	0.2	0.5
CV	0.8%	3.1%	2.9%

Between Run studies were done using a control run in replicate on eight (8) gels. N = 128

	% MM	% MB	% BB
Mean	73.7	7.1	19.2
SD	1.8	0.7	1.4
CV	2.4%	9.6%	7.1%

LINEARITY

The system has been validated to be linear to 1000 IU/L total CK using a UV kinetic method at 37°C.

SENSITIVITY

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

CORRELATION

Fifty (50) specimens, including 20 normals and 30 abnormal, were tested using both the REP and REP 3.

$$N = 50 \quad Y = 0.999X + 0.100$$

$$\text{Slope} = 0.999 \quad X = \text{CK-30 gels run}$$

$$\text{Intercept} = 0.100 \quad \text{on REP System}$$

$$r = 1.000 \quad X = \text{CK-16 gels run}$$

$$\text{on REP 3 System}$$

INTERPRETATION OF RESULTS

CK-MM

1. Often the only isoenzyme of CK found in normal serum.¹

2. 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 0-4%.²³ Note that although 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 setting.²
 - 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}
- 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 tumors.¹ (See Limitations note.)
- 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 hyperpyrexia.¹ (See Limitations note.)

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 identification.³⁰⁻³² Mitochondrial CK migrates cathodically to CK-MM³³, and a band designated "macro" CK, isolated from a cancer patient, also migrated cathodic to CK-MM.³⁴ (See Limitations note.)

Shaded areas indicate that text has been modified, added or deleted.

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REP CK-16 ISOENZYME SYSTEM

REP CK-16 Isoenzyme Kit **Cat. No. 3071**

- REP CK-16 Isoenzyme Gels (10)
- REP CK-16 Isoenzyme Reagent (10 x 700 µL)
- REP CK Isoenzyme Diluent (1 x 15 mL)
- REP Blotter A (10)
- REP Sample Cups (160 cups)

Other Supplies and Equipment

The following items, needed for performance of the REP CK-16 Isoenzyme Procedure, must be ordered individually.

Item	Cat. No.
REP (Rapid ElectroPhoresis Analyzer)	1352
REP 3	3700
REP CK/LD Isoenzyme Control (5 x 2.0 mL)	3073
REP Prep Solution	3100
SUREprep	1574
REP Prepper	1359

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