HISCL™ M2BPGi™ Assay Kit

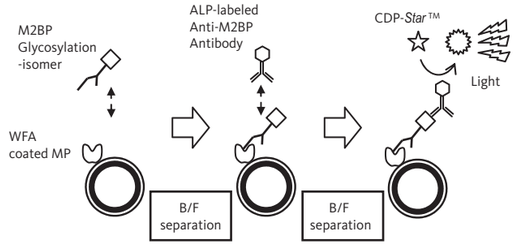
Identification of the IVD reagent
HISCL™ M2BPGi™ Assay Kit

Intended use
For In Vitro Diagnostic Use
Measurement of glycosylation isomer of Mac-2 binding protein (M2BPGi) in serum.

Development process and characteristics
This kit measures glycosylation isomer of Mac-2 binding protein based on the chemiluminescence enzyme immunoassay method with CDP-*Star*™ chemiluminescent substrate.[1]-[7]
This kit is exclusively designed for Sysmex automated immunoassay system.

Principles of the examination method
This kit measures M2BPGi based on the 2-step sandwich chemiluminescent enzyme immunoassay.
1. The sample is diluted with R1 reagent.
2. WFA-coated MP(magnetic particles) in R2 reagent specifically reacts with glycosylation isomer of M2BP in sample.
3. After B/F separation,ALP (alkaline phosphatase)-labeled anti-M2BP monoclonal antibodies (mouse) in R3 reagent specifically bind to M2BPGi on MP.
4. After B/F separation, ALP on MP decomposes CDP-*Star*™ substrate in R5 to an excited intermediate, which produces a luminescent signal.

Since the strength of this signal is proportional to M2BPGi value, an evaluation is possible based on the cut-off value established with a sample that contains a known value of M2BPGi (HISCL M2BPGi Calibrator)



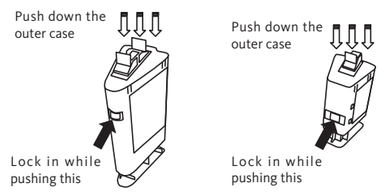
Components
This kit consists of the following reagents. Reagents 4 - 6 are separately sold products.
1. R1 reagent
2. R2 reagent: contains magnetic particles coated with WFA 5 mg/mL
3. R3 reagent: contains ALP-labeled anti-M2BP monoclonal antibodies (mouse) 0.1 U/mL
4. HISCL Substrate Reagent Set
   (1) R4 reagent
   (2)R5 reagent: contains CDP-*Star*™:
   Disodium 2-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)-tricyclo[3.3.1.1^{3,7}]decan}-4-yl)-1-phenyl phosphate 0.48mM
5. HISCL Washing solution
6. HISCL M2BPGi Calibrator
   (1) HISCL M2BPGi NC
   (2)HISCL M2BPGi PC

[Note 1] R1 reagent and R3 reagent are provided in a two-in-one reagent container.

Warnings and precautions
1. Use the kit according to the method stipulated in the package insert. The reliability of results cannot be guaranteed if the kit is used with a method or for a purpose other than those stipulated.
2. Handle each reagent carefully without generating air bubbles, which may produce incorrect results of analysis. If bubbles appear, wait until they disappear.
3. Do not combine reagents from different kits. Do not pool reagents even if the lot numbers of kits are the same. Use reagents prior to the expiry date. The reliability of results cannot be guaranteed if reagents are used past their expiration date.
4. Avoid contact of the R5 reagent with the skin and eyes, since it is an alkaline solution with pH9.6.
5. All calibrator bottles should be quickly closed after dropping calibrator solution, and then stored at 2-8 °C. If bottles are left open, calibrators may become concentrated by evaporation, resulting in incorrect calibration.
6. When taken out of the reagent holder of the analyzer, store R1-R3 reagents at 2-8°C. Stir the R2 reagent according to [Examination procedure] just before you return it to the analyzer. Do not use reagents once they have been frozen, since they may exhibit deterioration.
7. The Calibration is valid for 30 days. However, even within this period, calibrate again in the following cases:
   ・When new R1-R3 reagents are used with another Lot No.
   ・When quality assurance results are abnormal.
   ・After specified maintenance and/or repair of the analyzer (see analyzer instruction manual).
8. R1 to R4 reagents and Calibrator contain sodium azide. Since sodium azide reacts with lead tubing and copper tubing to generate metal azides which can explode, use a large quantity of water when disposing it. In case of contact with the eyes, mouth, or hands, perform emergency treatment such as washing with a large quantity of water. If necessary, consult a physician.
9. Handle samples carefully. They sometimes contain HBV, HCV, HIV, etc.
10. Do not use the reagent bottles, etc. for other purposes.
11. Use only the reagents (R1-R5 reagents, Calibrators and Washing solution) specified in this package insert.
12. Be certain to assemble the reagent containers according to [Examination procedure]. Incorrectly assembled containers may result in device errors or cause evaporation of reagents.
13. Install the R4 reagent and R5 reagent carefully to prevent contamination by alkaline phosphatase in saliva or on skin. To prevent absorption of excess CO_2, do not remove R5 reagent from the instrument until its bottle is empty and requires replacement.

Examination procedure
1. Preparation for measurement
   (1) Gently mix R2 reagent thoroughly by circling the container. See and confirm the magnetic particles have mixed uniformly.

   (2)First of all push down the outer cases of reagent containers firmly to tear the aluminum seals on the inner bottles.



REF	Catalogue number		Use by
IVD	In vitro diagnostic medical device	LOT	Batch code
	Manufacturer	Σ	Sufficient for
	Consult instructions for use		
	Temperature limitation		

(3) Set the containers at the indicated position of the analyzer.
(4) As a rule dispense 200µL of the sample to reduce the possible effects of evaporation. Refer to the analyzer instruction manual for the minimum volume.

2. Standard assay method *
(1) Dispense 10µL of the sample and 50µL of R1 reagent into a reaction cuvette, and then incubate for 2 minutes at 42°C.
(2) Dispense 30µL of R2 reagent into the cuvette, incubate for 1 minutes at 42°C, and then perform magnet separation (contact the magnet with the cuvette, and aspirate liquid).
(3) Dispense 100-700µL of Washing solution, and then perform magnetic separation. Repeat this procedure 3 times.
(4) Dispense 100µL of R3 reagent into the cuvette, incubate for 2.5 minutes at 42°C, and then perform magnetic separation (contact the magnet with the cuvette, and aspirate liquid).
(5) Dispense 100-700µL of Washing solution, and then perform magnetic separation. Repeat this procedure 3 times.
(6) Dispense 50µL of R4 reagent and mix, dispense 100µL of R5 reagent and mix, incubate for 5 minutes at 42°C, and then measure light intensity.

3. Setting cut-off value
(1) Gently stir HISCL M2BPGi NC and HISCL M2BPGi PC without generating bubbles. Position them according to the analyzer instruction manual.
(2) Carry out procedures according to the "standard assay method", and then measure light intensity.
(3) Set the cut-off value based on the light intensity in HISCL M2BPGi PC. *

Cut-off value = (P)
Where:
P is the light intensity in HISCL M2BPGi PC

4. Sample measurement
(1) Position a sample according to the analyzer instruction manual.
(2) Carry out procedures according to the "standard assay method", and then measure light intensity.
(3) Calculate cut-off Index (C.O.I.) of sample. *

Cut-off index (C.O.I.) = (S-N)/(C-N)
Where:
S is the light intensity in sample
C is the cut-off value
N is the light intensity in HISCL M2BPGi NC

* The analyzer automatically carries out these procedures.

Storage and shelf life after first opening
Store at 2-8°C. The shelf life is 30 days after opening.

Control procedure
Analyze control materials as samples according to [Examination procedure].

Interpretation of results
The sample is judged positive(1+) when the light intensity is above the cut-off value (1.00 ≤ C.O.I. < 3.00), positive(2+) when the light intensity is above the cut-off value (C.O.I. ≥ 3.00), and negative(-) when the light intensity is below the cut-off value (C.O.I. < 1.00).

[Note 2]
1. This product is intended for in vitro differential diagnosis of non-chronic hepatitis (-), chronic hepatitis (1+), and cirrhosis (2+) based on the M2BPGi level as evidenced by results from a multicenter clinical study of assay performance. The assay results of this kit show good agreement with liver fibrosis stages as assessed by using liver biopsy. However, diagnosis should be made comprehensively on the basis of other relevant test results and clinical symptoms since the result should rarely differ between this product and liver biopsy.
2. Since the primary diseases of liver fibrosis may influence the result, a comprehensive diagnosis should be made on the basis of other relevant test results and clinical symptoms.
3. This product has not been evaluated for in vitro diagnostic use to detect fibrosis in other organs. Therefore, a diagnosis should be made comprehensively on the basis of other relevant test results and clinical symptoms.
4. It is generally known that immunoassays can have false positive and negative results due to nonspecific reactions. Therefore, a diagnosis should be made comprehensively on the basis of other relevant test results and clinical symptoms. Nonspecific reactions are caused by autoantibodies, insoluble substances (fibrin, in particular), and natural antibodies.
5. It has been demonstrated that the assay can show false positivity when samples are obtained from patients with cancer. Therefore, a diagnosis should be made comprehensively on the basis of other relevant test results and clinical symptoms since samples from patients with cancer may yield false-positive results.
6. The kit can report false-positive results if samples are obtained from patients with pre-existing nephrotic syndrome. Therefore, a diagnosis should be made comprehensively on the basis of other relevant test results and clinical symptoms.

Performance characteristics
1. Sensitivity
When HISCL M2BPGi NC and HISCL M2BPGi PC are analyzed 5 times, the result should be $(X_{NC}+2SD_{NC})<(X_{PC}-2SD_{PC})$ when the mean light intensities are X_{NC} and X_{PC} and their standard deviations are SD_{NC} and SD_{PC} respectively.
2. Accuracy
(1) When an M2BPGi negative control serum is analyzed, the result is negative.
(2) When an M2BPGi positive control serum is analyzed, the resulting value is within the known value ±30%.
3. Reproducibility
(1) When an M2BPGi negative control serum is analyzed simultaneously 10 times, the result is negative.
(2) When an M2BPGi positive control serum is analyzed simultaneously 10 times, the CV is ≤ 15%.
4. Measurement range
C.O.I. of 0.10 to 20.00

[Note 3] Counts :
Unit of light intensity on Sysmex automated immunoassay system.
[Note 4] M2BPGi negative control serum: C.O.I. ≤ 0.60
M2BPGi positive control serum: C.O.I. = 2.70-3.30

Limitations of the examination procedure
Interference
1. Hemoglobin(500 mg/dL or lower), bilirubin (bilirubin F: 18.5 mg/dL or lower, bilirubin C: 20.2 mg/dL or lower), chylomicrons (1,560 formazine turbidity units or lower) and RF(550 IU/mL or lower) each have almost no effect on measurements.
2. Cloudy or hemolyzed sample may cause incorrect determination.

Reagent preparation
All reagents are ready-to-use.

Primary sample collection, handling and storage
Human serum
1. If samples must be stored, freeze at -20°C or lower. Do not repeat freezing and thawing of samples, which may induce formation of particulates and cause incorrect results.
2. Fibrin-clotted samples should be centrifuged at 2,000xg for 10 minutes to remove insoluble matter.
3. Please extract serum not to hemolyze.

Disposal procedures
1. Incinerate used sample tubes or reagent bottles, or dispose of them as medical waste or industrial waste according to the rules stipulated for waste materials.
2. When apparatus that has come in contact with any specimens is sterilized, perform sterilization using one of the following methods:
・Immerse in 0.05% formalin solution at 37°C for 72 hours or longer.
・Immerse in 2% glutaraldehyde solution for 1 hour or longer.
・Immerse in a solution containing 0.1% or more sodium hypochlorite for 1 hour or longer.
・Autoclave at 121°C for at least 1 hour.

Literature references
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Product information
HISCL M2BPGi Assay Kit For 100 Tests

Traceability of values assigned to calibrators
HISCL M2BPGi PC has been adjusted by in-house standard materials.

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01/2016

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