Destina Genomics®

LiverAce™ SinglePlex Kit

Liver Injury

miR-122 Test

96-Well Plate Assay

Ref. CLA2K-96-2024

User Guide

LiverAce™ SinglePlex Kit

miR-122

96-Well Plate Assay

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For Research Use Only. Not for Use in Diagnostic Procedures.

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex® Corporation ('Luminex®'), you, the customer, acquire the right under Luminex®'s patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with Luminex®'s laser based fluorescent analytical test instrumentation marketed under the name of Luminex® 100™ IS, 200™, HTS, FLEXMAP 3D®, INTELLIFLEX®, MAGPIX®.

Introduction

Drug toxicity is the leading cause of acute liver failure in the worldwide. Patients with liver damage generally display elevated amounts of circulating microRNA122-5p (miRNA122) in serum; this miRNA can serve as biomarker of drug-related liver toxicity. Monitoring this biomarker can greatly help clinicians avoid drug-induced liver failure. Performing laboratory tests to characterize the side effects of potential therapeutics is an essential part of drug development.

For different liver diseases in humans, miRNA122 has received regulatory support for further qualification by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Importantly, has been shown to be a more sensitive and specific biomarker for liver toxicity when compared with standard protein biomarkers. For example, circulating miRNA122 accurately reports human Drug Induced Liver Injury (DILI) after acetaminophen overdose at first presentation to hospital at a time when current markers, such as ALT/AST, are still within normal ranges, providing earlier and more accurate information on liver damage than ALT/AST testing.

The LiverAce™ kit offers a unique testing of miRNA122 across a wide range of liver disease states and species. You can rely on the quality that we build into each kit to produce results you can trust. In addition to the assay characteristics listed in the protocol, other performance criteria evaluated during the validation process include: cross-reactivity, dilution linearity, kit stability, and sample behavior (e.g. detectability and stability).

LiverAce™ can directly analyse with high specificity circulating miRNA122 in biofluids. Current complex protocols for detecting miRNA122 (short RNA extraction/isolation, reverse transcription, amplification steps and analysis) with inherent variability and high cost to obtain results has limited its development as a diagnostic biomarker in liver injury and drug toxicity testing. Once the analyte miRNA122 of interest has been identified, you can rely on the quality that we build into each kit to produce results you can trust.

Each kit meets stringent manufacturing criteria to ensure batch-to-

batch reproducibility. The LiverAce™ kit thus enables you to direct detect and quantify miRNA122 by merging with the Luminex® xMAP® platform in a magnetic bead format.

LiverAce™ offers you the ability to accurately analyse circulating miRNA122 in serum and/or plasma samples, without the need of extracting RNAs, generating cDNA and amplification steps.

For research use only. Not for use in diagnostic procedures. Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

Principle

LiverAce™ is based on the Luminex® xMAP® technology — one of the fastest growing and most respected bead-based technology offering applications throughout the molecular diagnostic and capable of profiling levels of miRNA122 in biological fluids.

- Luminex® uses proprietary techniques to internally color-code microspheres.
- After miRNA122 from a test sample is captured by the miRNA122 Bead, the SMART-C is introduced.
- The reaction mixture is then incubated with Streptavidin-Pycoerythrin conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- Each individual microsphere is identified and the result of its bioassay is quantified based on fluorescent reporter signals.

LiverAce™ combines the streamlined data acquisition power of Luminex® xPONENT® acquisition software with sophisticated analysis, integrating data acquisition and analysis seamlessly with all Luminex® instruments. The following Luminex® instruments can be used to acquire and analyze data using two detection methods:

- The Luminex® analyzers, Luminex® 200™, FLEXMAP 3D®, and xMAP® INTELLIFLEX, are flow cytometry-based instruments that integrate key xMAP® detection components, such as lasers, optics, advanced fluidics and high-speed digital signal

processors.

- The Luminex® analyzer (MAGPIX®), a CCD-based instrument that integrates key xMAP® capture and detection components with the speed and efficiency of magnetic beads.

Storage Conditions Upon Receipt

Kit components should be stored at 2-8 °C.**DO NOT** FREEZE miRNA122 Bead, SMART-C and Streptavidin-Phycoerythrin.

Reagents Supplied

Reagent	Volume	Quantity
96-Well Plate	-----	1 plate
miRNA122 Bead (R019)	70 µL	1 vial
miRNA122 Standard	Powder	1 vial
Assay Matrix	1.5 mL	1 vial
Assay Buffer	15 mL	1 bottle
Lysis Buffer (without additives)	12 mL	1 bottle
Additive 1	Powder	1 vial
Additive 2	30 µL	1 vial
10X Wash Buffer	25 mL	1 bottle
SMART-C	130 µL	1 vial
Reducing Agent	Powder	1 vial
500X Streptavidin-Phycoerythrin	15 µL	1 vial

Materials Required (not included)

Reagents

- MAGPIX® Drive Fluid PLUS, xMAP® Sheath Fluid PLUS or xMAP® Sheath Concentrate PLUS
- MilliQ water and DNase/RNase-free water

Instrumentation / Materials

- Pipettes with tips capable of delivering 2 µL to 1000 µL
- Multichannel pipettes capable of delivering 5 µL to 50 µL, or 25 µL to 200 µL
- Reagent reservoirs
- Polypropylene microcentrifuge tubes
- Aluminum foil
- Absorbent pads
- Laboratory vortex mixer
- Sonicator (Branson Ultrasonic Cleaner Model B200 or equivalent)
- Titer plate shaker (Lab-Line Instruments Model No. 4625 or equivalent)
- Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® instrument with xPONENT® software, or xMAP® INTELLIFLEX® instrument with INTELLIFLEX software by Luminex® Corporation
- Handheld Magnetic Separation Block

Safety Precautions

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium azide has been added to some reagents as a preservative. Although the concentrations are low, Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.
- The sodium cyanoborohydride (Reducing Agent) is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200). Poison. Danger. Corrosive. Flammable solid. Water reactive. Hygroscopic. May be fatal if swallowed, inhaled or absorbed through skin. Causes burns to any area of contact. Follow universal precautions for handling.

Note: See Full Labels of Hazardous components on next page.

Full labels of hazardous components in this kit:

Ingredient	Label	
miRNA122		**Warning.** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Assay Matrix		**Warning**. Harmful if swallowed. Harmful to aquatic life with long lasting effects. Avoid release to the environment.
10X Wash Buffer		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
Streptavidin-Phycoerythrin		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Reducing Agent		**Warning.** In contact with water releases flammable gases which may ignite spontaneously. Harmful if swallowed. Fatal in contact with skin. Harmful if inhaled. Causes serious eye irritation. Very toxic to aquatic life with long lasting effects.

Technical Guidelines

To ensure reliable and reproducible results, the operator should carefully read this entire SOP and fully understand all aspects of each step before running the assay. The following notes should be reviewed and fully understood before setting up the assay.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The capture beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with an opaque plate lid or aluminum foil during all incubation steps.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- The standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at ≤ -20 °C for 1 month and at ≤ -80 °C for greater than one month.
- The 50 mM Reducing Agent solution can be used within 30 min or alternatively stored, as well as the resuspended stock, at -20°C for up to one year. Avoid multiple (>1) freeze/thaw cycles. Discard after single use.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.

- This protocol was developed using the Hand-Held Magnetic Plate Washer (Cat. No. A14179, Thermo Fisher) and the 96-Well Conical Bottom Plate (Cat. No. 249944, Thermo Fisher). Other washer-plate combinations should be validated by the end user.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay, adjust probe height according to the protocols recommended by Luminex®.
- When using the 96-Well Conical Bottom Plate provided in the kit, the final resuspension should be with 120 µL Wash Buffer in each well and 100 µL should be aspirated.
- Vortex all reagents well before adding to plate.

Sample Collection and Storage

Preparation of Samples

- Avoid multiple (> 1) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.

Note:

- A total of 25 µL per well of sample is used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

Preparation of reagents

A) Additive 1

Centrifuge the vial containing Additive 1 (powder) before opening it[1]. Reconstitute Additive 1 with Lysis Buffer (without additives)[2], see vial for resuspension volume. Gently invert the vial multiple times to ensure thorough mixing, and if necessary, vortex the solution. In case of any aggregates, sonication can be employed to disperse them. The freshly prepared Additive 1 can be utilized during one week if proper storage at 2-8 ºC.

[1] *Additive 1 is prone to oxidation upon exposure to air. Although the material exhibits slight hygroscopic properties, it should be noted that when stored at room temperature, Additive 1 rapidly loses its reducing capability.*

[2] ***Lysis Buffer tends to precipitate.*** *Please ensure that there is no precipitate present before adding Additive 1.*

B) Lysis Buffer (with additives)

Add 9480 µL of Lysis Buffer (without additives) to an appropriate vial (not provided), then add 500 µL of Additive 1 and 20 µL of Additive 2. Invert the vial several times to mix.

C) Wash Buffer 1X

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 24 mL of 10X Wash Buffer with 216 mL deionized water. Store the unused portion at 2-8 °C for up to one month.

D) miRNA122 Bead

Vortex the individual vial of miRNA122 Bead for 30 seconds; sonicate for 30 seconds.

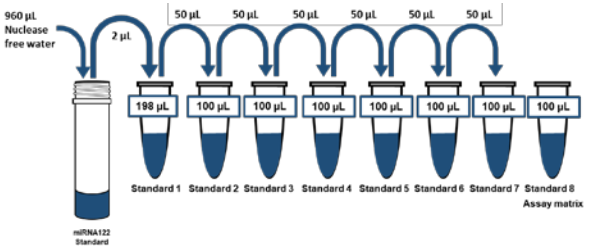
Add 8195 µL of Lysis Buffer (with additives) to an appropriate vial (not provided). Then add 55 µL from the miRNA122 Bead vial.

E) Preparation of miRNA122 Working Standards

Prepare the miRNA122 Working Standards on ice right before use. Prior to use, reconstitute the miRNA122 Standard with 960 µL of

nuclease-free water. Invert the vial several times and vortex for 10 seconds to mix well. The unused portion of the resuspended miRNA122 Standard stock may be stored at -20 °C for up to one month.

1. Label 8 polypropylene microfuge tubes Standard 1 through Standard 8.
2. Add 198 µL of Assay Matrix into the Standard 1 tube.
3. Add 150 µL of Assay Matrix into Standard 2 to Standard 7 tubes.
4. Add 2 µL of miRNA122 Standard to the Standard 1 tube, mix well and;
5. Transfer 50 µL of Standard 1 to the Standard 2 tube, mix well and;
6. Transfer 50 µL of Standard 2 to the Standard 3 tube, mix well and;
7. Repeat step 6 for Standard 4 to 7 tubes, changing pipette tips between dilution steps.
8. Add 150 µL of Assay Matrix to the Standard 8 tube to serve as background (0 pg/mL).



Standard	miRNA122 (pg/mL)
Standard 1	15000
Standard 2	5000
Standard 3	1667
Standard 4	556
Standard 5	185
Standard 6	62
Standard 7	21
Standard 8	0

F) Reducing Agent

Quick centrifuge vial prior to opening. Reconstitute Reducing agent with Milli-Q water to 1M solution (see vial for resuspension volume). Invert the vial several times to mix and vortex for 30 seconds. Prepare a 50 mM reducing agent solution (i.e. 50 µL 1 M Reducing Agent + 950 µL Milli-Q water). Keep 50 mM solution in ice and use within 30 min or alternatively store at -20°C for up to one year. Avoid multiple (>1) freeze/thaw cycles. Discard after single use.

G) LiverAce™ Mix

The LiverAce™ Mix contains SMART-C and Reducing Agent in Assay Buffer

Add 5300 µL of Assay Buffer to an appropriate vial (not provided), then add 110 µL of SMART-C and 110 µL of 50 mM Reducing Agent. Vortex the mixture for 30 seconds. Once the mix is prepared it must be used within 2-3 minutes.

H) Streptavidin-R-Phycoerythrin (SA-PE)

Centrifuge the vial containing SA-PE before opening it. Add 5988 µL of Assay Buffer to an appropriate vial (not provided), then add 12 µL

from the SA-PE vial. Vortex the mixture for 30 seconds. Once the mix is prepared it must be used within 2-3 minutes.

LiverAce™ Protocol

Note: Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.

Note: Allow all reagents to warm to room temperature (20-25ºC) before use in the assay, unless noted in the Preparation of Reagents section.

Note: It is recommended to run the assay in triplicate.

1. Vortex the diluted miRNA122 Bead and add 75 µL to each well.
2. Add 25 µL of each miRNA122 Standard (Standard 1 to Standard 8) into the standards wells.
3. Add 25 µL of Sample to the sample wells.
4. Seal and cover the plate, and incubate with agitation on a plate shaker for 2 hours at 30°C.
5. Wash plate 3 times following the instructions listed in the Plate Washing section
6. Add 50 µL of LiverAce™ Mix into each well.
7. Seal and cover the plate and incubate with agitation on a plate shaker for 1 hour at 40°C.
8. Wash plate 3 times following the instructions listed in the Plate Washing section.
9. Add 50 µL of SA-PE to each well.
10. Seal and cover the plate, and incubate with agitation on a plate shaker for 30 minutes at 30°C.
11. Wash plate 3 times following the instructions listed in the Plate Washing section
12. Add 120 µL of Wash Buffer to all wells. Resuspend the beads on a plate shaker for 5 minutes.
13. Run plate on Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® instrument with xPONENT® software or xMAP® INTELLIFLEX instrument with INTELLIFLEX software

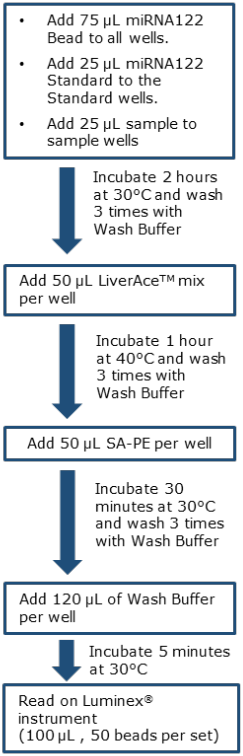


Plate Washing

Note: This protocol was developed using the Hand-Held Magnetic Plate Washer (Cat. No. A14179, Thermo Fisher) and the 96-Well Conical Bottom Plate (Cat. No. 249944, Thermo Fisher). Other washer-plate combinations should be validated by the end user.

Add 100 µL of Wash Buffer 1X to each well and place the plate on the magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 µL of Wash Buffer 1X by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps twice.

Equipment Settings

Luminex®, 200™, HTS, FLEXMAP 3D®, MAGPIX® instruments with xPONENT® software and xMAP® INTELLIFLEX instrument with INTELLIFLEX software:

These specifications are for the above listed instruments and software. Luminex® instruments with other software (for example, MasterPlex®, StarStation, LiquiChip, Bio-Plex® Manager™, LABScan™100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex® magnetic beads.

For magnetic bead assays, each instrument must be calibrated and performance verified with the indicated calibration and verification kits.

Note: When setting up a Protocol using the xPONENT® software, you must select MagPlex® as the Bead Type in the Acquisition settings.

Note: These assays cannot be run on any instruments using Luminex® IS 2.3 or Luminex® 1.7 software.

The Luminex® probe height must be adjusted to the plate provided in the kit.

Events	50 per bead	
Sample Size	100 µL	
Gate Settings	8,000 to 15,000	
Reporter Gain	Default (low PMT)	
Time Out	60 seconds	
Bead Set	Singleplex Beads	
	miRNA122	19

Assay Characteristics

Cross-Reactivity

There was no or negligible cross-reactivity between the miRNA122 Bead for other analytes present in body fluids.

Assay Sensitivity

Limit of Detection (LOD) and Lowest Limit of Quantification (LLOQ) are calculated using Belysa® software.

Analyte	LOD (pg/mL)	LLOQ (pg/mL)
miRNA122	2.4	21

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of analytes in a single assay. Inter-assay precision is generated from the mean of the %CV's across two different concentrations of analytes across 7 different assays.

Analyte	Intra-assay %CV	Inter-assay %CV
miRNA122	< 10%	< 20%

Accuracy

Spike Recovery: The data represent mean percent recovery of spiked standards ranging from low, medium, and high concentration in serum matrices (n=5).

Analyte	% Recovery in Assay Matrix
miRNA122	< 115%

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose. The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex® Corporation ("Luminex®"), you, the customer, acquire the right under the Luminex® patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with the Luminex® laser based fluorescent analytical test instrumentation marketed under the name of Luminex® 200™, HTS, FLEXMAP 3D®, xMAP® INTELLIFLEX, MAGPIX®.

Contact Information and Technical Assistance

Go to https://destinagenomics.com/contact/.

Well Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1	Standard 1	Standard 1	Sample 1	Sample 1	Sample 1	Sample 9	Sample 9	Sample 9	Sample 17	Sample 17	Sample 17
B	Standard 2	Standard 2	Standard 2	Sample 2	Sample 2	Sample 2	Sample 10	Sample 10	Sample 10	Sample 18	Sample 18	Sample 18
C	Standard 3	Standard 3	Standard 3	Sample 3	Sample 3	Sample 3	Sample 11	Sample 11	Sample 11	Sample 19	Sample 19	Sample 19
D	Standard 4	Standard 4	Standard 4	Sample 4	Sample 4	Sample 4	Sample 12	Sample 12	Sample 12	Sample 20	Sample 20	Sample 20
E	Standard 5	Standard 5	Standard 5	Sample 5	Sample 5	Sample 5	Sample 13	Sample 13	Sample 13	Sample 21	Sample 21	Sample 21
F	Standard 6	Standard 6	Standard 6	Sample 6	Sample 6	Sample 6	Sample 14	Sample 14	Sample 14	Sample 22	Sample 22	Sample 22
G	Standard 7	Standard 7	Standard 7	Sample 7	Sample 7	Sample 7	Sample 15	Sample 15	Sample 15	Sample 23	Sample 23	Sample 23
H	Standard 8 0 pg/mL (blank/blank)	Standard 8 0 pg/mL (blank/blank)	Standard 8 0 pg/mL (blank/blank)	Sample 8	Sample 8	Sample 8	Sample 16	Sample 16	Sample 16	Sample 24	Sample 24	Sample 24

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