User Guide

LiverAce™ Singleplex Kit

PCR-free miR-122 detection

96-Well Plate Assay

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

Introduction

Hepatotoxicity is a primary cause of late-stage drug attrition, costing the pharmaceutical industry up to $3 Billion annually. Performing laboratory tests to accurately characterize hepatotoxicity is an essential and critical part of drug development.

Traditional testing relies on indicators like ALT/AST, which lack specificity and are often released by non-hepatic tissues, leading to costly false safety flags. To overcome these limitations, the industry is shifting towards advanced molecular biomarkers.

During drug-induced liver damage, pre-clinical models and human subjects exhibit early and significant elevations of circulating miR-122 in serum and plasma. Unlike traditional enzymatic indicators, miR-122 provides a highly specific, early signal that prevents these false safety flags. Monitoring this biomarker enables pharmaceutical developers and Contract Research Organizations (CROs) to accurately identify toxicity problems early in the pipeline, allowing for confident, "Fail-Fast" Go/No-Go decisions.

However, standard PCR-based methods are inherently limited by amplification bias, and are fundamentally blind to miRNA degradation, missing the vast majority of the true miR-122 isomiR pool.

The LiverAce™ Singleplex Kit is a Research Use Only (RUO) assay designed to overcome these analytical barriers. Powered by our platform-agnostic Destina Chemistry, LiverAce™ Singleplex enables the direct, extraction-free, and PCR-free quantification of miR-122 and its isomiRs. By delivering absolute isomiR resolution with a simple immunoassay-like workflow, this kit provides accurate, reproducible, and scalable hepatotoxicity assessment across all stages of the drug development lifecycle.

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

The LiverAce™ Singleplex Kit combines the molecular precision of Destina Chemistry (DGL-Tech) with the operational scalability of the Luminex® xMAP® platform. By utilizing standard immunoassay workflows, this synergistic approach transforms existing platforms into high-value molecular assays, completely bypassing the limitations and variability of enzymatic amplification.

- **Step 1: Target Capture (PCR-Free).** The assay utilizes a proprietary, patented dual-recognition mechanism. A specific DGL-Probe, immobilized on a magnetic Luminex® microsphere, captures the miR-122 target, including all its isomiR variants, directly from the biofluid without the need for prior RNA extraction.

- **Step 2: Dynamic Chemical Labeling.** Upon hybridization, a unique "Chemical Pocket" is formed. A Biotinylated SMART-Base is then introduced and covalently locked into this pocket with single-nucleotide specificity, ensuring 100% accurate sequence recognition and eliminating false positives.

- **Step 3: Reporter Binding & Readout.** The reaction mixture is incubated with a Streptavidin-Phycoerythrin (SA-PE) conjugate, which binds to the Biotinylated SMART-Base to serve as the fluorescent reporter.

- **Step 4: Data Acquisition.** The Luminex® analyzer reads the unique internal color code of each magnetic bead to identify the analyte, while simultaneously measuring the phycoerythrin reporter signal to precisely quantify the miR-122 concentration.

LiverAce™ Singleplex seamlessly integrates with the XPONENT® acquisition software across all compatible Luminex® instruments, including the Luminex® 200™, FLEXMAP 3D®, xMAP® INTELLIFLEX, and MAGPIX® analyzers. This provides toxicology labs with unmatched data certainty, leveraging existing infrastructure to deliver superior insights.

Storage Conditions Upon Receipt

Kit components should be stored at 2-8 °C.

DO NOT FREEZE.

Reagents Supplied

Reagent	Cat. No.	Volume
96-Well Plate	RAM001	-----
miR-122 Beads	RAM002	70 µL
miR-122 Standard	RAM003	Powder
miR-122 Control	RAM004	Powder
Assay Matrix	RAM005	1.5 mL
Assay Buffer	RAM006	15 mL
Lysis Buffer	RAM007	13 mL
Additive 1	RAM008	Powder
Additive 2	RAM009	30 µL
10X Wash Buffer	RAM010	115 mL
SMART-C-Biotin	RAM011	130 µL
Reducing Agent	RAM012	Powder
500X Streptavidin-Phycoerythrin	RAM013	15 µL

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)
- Incubating microplate shaker (Fisher Scientific 15564070 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
- Magnetic Plate Separator Diasorin CN-0269-01 or LifeSep™ 96F Cat. No. Z740159

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.

- **Full labels of hazardous components in this kit:**

Ingredient	Label	
miR-122		**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.
- The microplate 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 700-800 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®.
- 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

The LiverAce™ Singleplex Kit is optimized for micro-sampling, requiring only 25 µL of sample per well. This low-volume requirement maximizes data output from precious pre-clinical models while maintaining direct translatability to human clinical trials.

General Guidelines:

- **Tube Compatibility:** All samples must be stored in polypropylene tubes. DO NOT STORE SAMPLES IN GLASS.
- **Sample Quality:** Avoid using samples with gross hemolysis, lipemia, or visible cellular debris. When using heparin as an anti-coagulant, strictly ensure a maximum of 10 IU heparin per mL of blood to prevent falsely high values.
- **Thawing:** When using frozen samples, thaw completely, vortex

vigorously, and centrifuge prior to assaying to remove any particulates

Sample Type Preparation:

- **Serum:** Allow blood to clot for at least 30 minutes before centrifugation (10 minutes at 1000 x g).
- **Plasma:** Collect in appropriate anti-coagulant tubes and centrifuge (10 minutes at 1000 x g).

Storage: Remove serum or plasma immediately after centrifugation. Assay immediately, or aliquot and store at -80ºC. Strictly avoid multiple (>2) freeze/thaw cycles to ensure biomarker integrity.

Preparation of reagents

A) 1x Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. **Note:** Concentrated wash buffer may precipitate during storage. If salt crystals are present, warm the buffer at 30°C–37°C and mix gently until all salts are completely back into solution before use.

Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water. Store the unused portion at 2-8 °C for up to one month.

Note for Automated Systems: *If using automatic dispensing ensure you prepare a sufficient volume of 1X Wash Buffer to account for instrument priming and dead volume*

B) Additive 1

Centrifuge the vial containing Additive 1 (powder) before opening it[1]. Reconstitute Additive 1 with 2mL of Lysis Buffer[2]. 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. If precipitation or cloudiness is observed, warm the buffer at 30°C–37°C and mix gently until the solution is completely clear.*

C) Lysis Buffer with additives

Add 9480 µL of Lysis Buffer 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.

D) miR-122 Beads

Vortex the individual vial of miR-122 Beads for 30 seconds; sonicate for 30 seconds.

Add 8940 µL of Lysis Buffer (with additives) to an appropriate vial (not provided). Then add 60 µL from the miR-122 Beads vial.

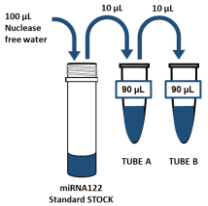
E) Preparation of miR-122 Standards

CRITICAL: Prepare Standards and Controls ON ICE RIGHT BEFORE USE.

Reconstitution: Prior to use, reconstitute the miR-122 Standard vial with 100 µL of nuclease-free water. Invert the vial several times and vortex for 10 seconds to mix well. (The unused portion of the resuspended miR-122 Standard stock may be stored at -20 °C for up to one month).

Intermediate Standards (Tubes A & B): Label 2 microfuge tubes and add 90 µL of Assay Matrix to each tube.

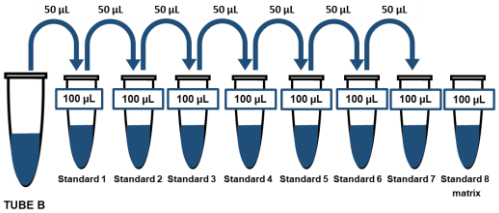
- *Tube A:* Transfer 10 µL of the miR-122 Standard STOCK to Tube A and mix well.
- *Tube B:* Transfer 10 µL from Tube A to Tube B and mix well. (Change pipette tips between tubes).



Working Standards (Serial Dilution): Label 8 polypropylene microfuge tubes (Standard 1 through Standard 8). Add 100 µL of Assay Matrix into Standard 1 to Standard 7 tubes.

- Transfer 50 µL of Tube B to the Standard 1 tube and mix well.
- Transfer 50 µL of Standard 1 to the Standard 2 tube and mix well. Continue this 1:3 serial dilution through Standard 7, changing pipette tips between dilution steps.

Background: Add 100 µL of Assay Matrix to the Standard 8 tube to serve as background (0 pg/mL).



Standard	miR-122 (pg/mL)
Standard 1	6000
Standard 2	2000
Standard 3	667
Standard 4	222
Standard 5	74
Standard 6	25
Standard 7	8
Standard 8	0

F) Preparation of miR-122 Controls

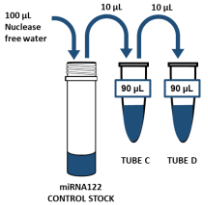
Prepare miR-122 Control working solutions on ice RIGHT BEFORE USE. Prior to use, reconstitute the miR-122 Control with 100 µL of nuclease-free water. Invert the vial several times and vortex for 10 seconds to mix well. The unused portion of the resuspended miR-122 Control stock may be stored at -20 °C for up to one month.

F.1) Preparation of **INTERMEDIATE controls**:

Label 2 microfuge tubes and add 90 µL of **Assay Matrix** on each tube.

Tube C: Transfer 10 µL of the **miR-122 Control STOCK** to Tube C. Mix well and change tips between tubes.

Tube D: Transfer 10 µL from Tube C to Tube D. Mix well.

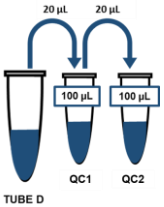


F.2) Preparation of **miR-122 Working Controls**

Label 2 additional polypropylene microfuge tubes and add 100 µL of **Assay Matrix** each.

Quality Control 1 (QC1): Transfer 20 µL of Tube D to QC1 tube. Mix well and change tips between tubes.

Quality Control 2 (QC2): Transfer 20 µL of the QC1 to QC2 tube. Mix well.



Analyte	QC level	Expected Range	Units
miR122	QC1	2100 - 3900	pg/mL
	QC2	350 - 650	pg/mL

G) Reducing Agent

Quick centrifuge vial prior to opening. Reconstitute Reducing agent with 500 µL of 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.

H) LiverAce™ Mix

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

Before use, add 5520 µL of Assay Buffer to an appropriate vial (not provided), then add 115 µL of SMART-C-Biotin and 115 µL of 50 mM Reducing Agent. Vortex the mixture for 30 seconds.

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

Before use, 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.

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 the reagents to warm to room temperature except for 500x Streptavidin-Phycoerythrin

Note: Run the assay in duplicate.

1. Prepare Wash buffer 1x as indicated in section A.
2. Prepare Additive 1 as indicated in section B.
3. Prepare Lysis buffer with additives as indicated in section C.
4. Prepare diluted miRNA122 Beads mix as indicated in section D
5. Prepare Standards and Controls as indicated in sections E and F and keep on ice.

6. Vortex the diluted miRNA122 Beads prepared in step 4 and add 75 µL to each well.

7. Add 25 µL of each miRNA122 Standard (Standard 1 to Standard 8) into the "Standards" wells.

8. Add 25 µL of miRNA122 Controls (QCs) to the "Controls" wells.

9. Add 25 µL of sample to each "Sample" well.

10. Seal, cover with foil and incubate with agitation on a plate shaker for 2 hours at 30°C, 700-800 rpm.

11. Wash the plate following the instructions listed in the Plate Washing section.

12. Prepare the reducing agent as indicated in section G.

13. Prepare LiverAce™ Mix as indicated in section H.

14. Add 50 µL of LiverAce™ Mix into each well.

15. Seal, cover with foil and incubate with agitation on a plate shaker for 1 hour at 40°C, 700-800 rpm.

16. Wash the plate following the instructions listed in the Plate Washing section.

17. Prepare SA-PE dilution as indicated in section I.

18. Add 50 µL of SA-PE to each well.

19. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at 30°C, 700-800 rpm.

20. Wash the plate following the instructions listed in the Plate Washing section.

21. Add 120 µL of Wash Buffer to all wells.

22. Cover with foil and incubate with agitation on a plate shaker for 5 minutes at 30°C, 700-800 rpm.

23. 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 optimized for manual washing using either of the following Magnetic Plate Separators: DiaSorin (CN-0269-01) or LifeSep™ 96F (Cat. No. Z740159). If an automated washing system is used, the protocol will require end-user optimization to prevent bead loss.

Manual Washing Procedure:

1. Add 100 µL of 1X Wash Buffer to each well.
2. Place the plate on the magnetic separator for 60 seconds to allow the magnetic beads to settle completely.
3. Remove the well contents by gently decanting the plate into an appropriate waste receptacle. Gently tap the inverted plate on clean absorbent pads to remove any residual liquid.
4. Remove the plate from the magnet.
5. Add 200 µL of 1X Wash Buffer to each well and shake for 30 seconds.
6. Reattach the plate to the magnet and let the beads settle for 60 seconds.
7. Remove the well contents as described in Step 3.
8. Repeat steps 4 through 7 two additional times.

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	3,000 to 20,000	
Reporter Gain	Default (low PMT)	
Time Out	60 seconds	
Bead Set	Singleplex Beads	
	miR-122	19

Assay Characteristics

Assay Sensitivity

Limit of Detection (LOD) and Limit of Quantification (LOQ) are calculated using data from 9 independent runs. LOD was calculated using the following formula: LOD = LoB + 1.625*SD (Std8 conc). The average LOD from 9 runs is presented. LOQ was calculated using the Analyse-it® software (Method Validation Edition). The software applies statistical approaches derived from CLSI recommendations for detection capability assessment. The calculations were performed using experimental replicate data generated (a total of 23 replicates per sample) across 9 assay runs.

Analyte	LOD (pg/mL)	LOQ (pg/mL)
miR-122	3.6	10.8

Precision

Intra-assay and Inter-assay precision are calculated using Analyse-it® software (Method Validation Edition), based on experimental replicate data (a total of 23 replicates per sample) across 9 assay runs.

Analyte	Intra-assay %CV	Inter-assay %CV
miR-122	< 20%	< 20%

Accuracy

Accuracy is evaluated based on the recovery of calibration standards and QC samples, calculated by comparing the measured concentration with the assigned concentration.

Analyte	% Recovery
miR-122	70 -130%

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

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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	QC1	QC1	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31
B	Standard 2	Standard 2	QC2	QC2	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32
C	Standard 3	Standard 3	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
D	Standard 4	Standard 4	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
E	Standard 5	Standard 5	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
F	Standard 6	Standard 6	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
G	Standard 7	Standard 7	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
H	Standard 8 (Background)	Standard 8 (Background)	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38