Destina Genomics®

LiverAce™ COMBO Kit

Liver Injury

miR-122 & CK18 Test

96-Well Plate Assay

Ref. CLA2K-96-2024

User Guide

LiverAce™ COMBO Kit
miR-122 & CK18

96-Well Plate Assay

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

Introduction

Drug toxicity is a major cause of severe liver damage worldwide, often resulting in acute liver failure. Patients with liver issues typically show increased levels of specific biomarkers in their blood, which can act as indicators of drug-related liver toxicity. Monitoring these biomarkers is crucial for clinicians to prevent drug-induced liver failure. Evaluating the side effects of potential treatments through laboratory tests is a fundamental step in drug development.

Current international efforts and scientific findings strongly suggest that an innovative approach involving a combination of biomarkers, including both proteins and miRNAs, could be optimal for identifying drug-induced liver injury (DILI) and predicting its progression. The search for effective, organ-specific toxicity protein-based biomarkers is complemented by the development of new assays to measure these critical indicators, such as Cytokeratin-18 (CK18). In addition to proteins, microRNA-122-5p (miR-122) in the bloodstream has gained regulatory support for further qualification from the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Importantly, miR-122 has demonstrated higher sensitivity and specificity as a biomarker for liver toxicity compared to standard protein biomarkers.

The LiverAce™ COMBO Kit provides an array of analytes covering a broad spectrum of disease states and species.

Every LiverAce™ COMBO Kit includes:

- A calibration curve for absolute quantification of the panel biomarkers.
- An optimized matrix that mimics the native analyte environment.
- An optimized lysis buffer for liberating miRNA from lipidic membranes and protein complexes.
- Detection molecules (antibody/SMART-C) cocktails designed for consistent analyte profiles within the panel.

Each panel and kit follow to stringent manufacturing criteria, ensuring batch-to-batch reproducibility. LiverAce™ COMBO Kit offers

a unique combination of testing for both K18 and miR-122, across a diverse range of liver disease states and species, ensuring reliable results with each kit. Utilizing the Luminex® xMAP platform in a magnetic bead format provides the benefits of optimal speed and sensitivity, enabling quantitative multiplex detection simultaneously, a feature that can significantly enhance productivity.

DESTINA's LiverAce™ COMBO Kits stands out as the most versatile system for liver toxicity research. Whether you are looking for single or multiplex biomarker solutions, we collaborate with you to design, develop, analytically validate, and construct the most extensive library for biomarker detection and quantitation.

For research use only. Not for use in diagnostic procedures.

Please read the entire protocol before use.

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

Principle

LiverAce™ COMBO products leverage the cutting-edge Luminex® xMAP technology, a highly regarded multiplex technology widely used in life sciences. This technology facilitates diverse bioassays conducted on fluorescent-coded magnetic beads.

Key features of Luminex® products include the internal color-coding of microspheres with two fluorescent dyes. By precisely modulating the concentrations of these dyes, distinct sets of colored 5.6 µm polystyrene microspheres are created, with a unique capture molecule.

Here's a step-by-step breakdown of the assay process:

1. Capture: Analytes from the test sample are captured by the microspheres.
2. Detection: A biotinylated detection molecule is introduced to the captured analytes.
3. Reaction Completion: The reaction mixture is incubated with Streptavidin-Phycoerythrin conjugate, the reporter

molecule, completing the reaction on the microsphere surface.

Luminex® instruments capable of acquiring and analyzing data via two detection methods include:

- Luminex® analyzers, such as Luminex® 200™, FLEXMAP 3D®, and xMAP INTELLIFLEX®. These instruments are flow cytometry-based, integrating crucial xMAP detection components.
- Luminex® analyzer (MAGPIX®), a CCD-based instrument combining xMAP capture and detection components with the efficiency of magnetic beads.

Individual microspheres are identified and bioassay results are quantified based on fluorescent reporter signals. Luminex® xPONENT® software seamlessly combines the power of data acquisition with all Luminex® instruments.

Storage Conditions Upon Receipt

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

DO NOT FREEZE miR-122 Bead, K18 Bead, SMART-C and Streptavidin-Phycoerythrin.

The K18 Standard must be stored at -20 °C immediately upon receipt of the kit.

Reagents Supplied

Reagent	Volume	Quantity
Set of two 96-Well Plates	-----	2 plates
K18 Bead (R018)	70 µL	1 vial
miR-122 Bead (R019)	70 µL	1 vial
Assay Matrix	1.5 mL	1 vial
Assay Buffer	25 mL	1 bottle
COMBO Lysis Buffer (without additives)	12 mL	1 bottle
Additive 1	Powder	1 vial
Additive 2	30 µL	1 vial
K18 Standard	Variable	1 vial
miR-122 Standard	Powder	1 vial
10X Wash Buffer	30 mL	1 bottle
K18 Detection	40 µL	1 vial
SMART-C	130 µL	1 vial
Reducing Agent	Powder	1 bottle
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 microfuge 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.

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

Ingredient	Label	
Standards		**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 anticoagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

Reagents Preparation

A) Additive 1

Centrifuge the vial containing Additive 1 (powder) before opening it[1]. Reconstitute Additive 1 with COMBO 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.] ***COMBO Lysis Buffer tends to precipitate.*** *Please ensure that there is no precipitate present before adding to Additive 1. It is recommended to heat in a water bath at 30°C until the precipitates are completely dissolved.*

B) COMBO Lysis Buffer (with additives)

Add 9230 µL of COMBO Lysis Buffer (without additives) to an

appropriate vial (not provided), then add 750 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 30 mL of 10X Wash Buffer with 270 mL deionized water. Store the unused portion at 2-8 °C for up to one month.

D) K18 Bead

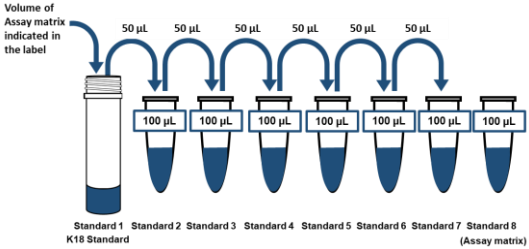
Vortex the individual vial of protein beads for 30 seconds; sonicate for 30 seconds.

Add 2695 µL of Assay Buffer to an appropriate vial (not provided). Then add 55 µL from the K18 Bead vial.

E) Preparation of Protein Working Standards

Prepare the Protein Working Standards on ice right before use.

1. Label 7 polypropylene microfuge tubes Standard 2 through Standard 8.
2. Add 100 µL of Assay Matrix into Standard 2 to Standard 7 tubes.
3. Add the volume of Assay Matrix written in the tube of the K18 Standard tube to prepare Standard 1, mix well and;
4. Transfer 50 µL of Standard 1 to the Standard 2 tube, mix well and;
5. Transfer 50 µL of Standard 2 to the Standard 3 tube, mix well and;
6. Repeat step 5 for Standard 4 to 7 tubes, changing pipette tips between dilution steps.
7. Add 100 µL of Assay Matrix to the Standard 8 tube to serve as background (0 pg/mL).



Standard	K18 (pg/mL)
Standard 1	100000
Standard 2	33333
Standard 3	11111
Standard 4	3704
Standard 5	1235
Standard 6	412
Standard 7	137
Standard 8	0

F) miR-122 Bead

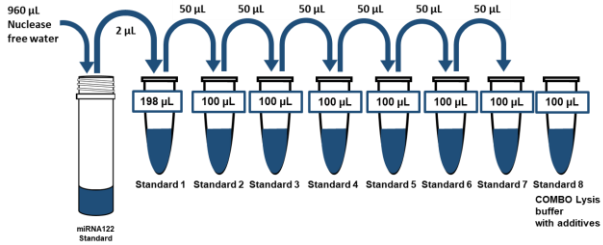
Vortex the individual vial of miR-122 Bead for 30 seconds; sonicate for 30 seconds.

Add 2695 µL of COMBO Lysis Buffer (with additives) to an appropriate vial (not provided). Then add 55 µL from the miR-122 Bead vial.

G) Preparation of miR-122 Working Standards

Prepare the miR-122 Working Standards on ice right before use. Prior to use, reconstitute the miR-122 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 miR-122 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 COMBO Lysis Buffer (with additives) into the Standard 1 tube.
3. Add 100 µL of COMBO Lysis Buffer (with additives) into Standard 2 to Standard 7 tubes.
4. Add 2 µL of miR-122 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 100 µL of COMBO Lysis Buffer (with additives) to the Standard 8 tube to serve as background (0 pg/mL).



Standard	miR-122 (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

H) 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.

I) LiverAce™ COMBO Mix A

The LiverAce™ COMBO Mix B contains the K18 Detection Antibody in Assay Buffer.

Add 5765 µL of Assay Buffer to an appropriate vial (not provided), then add 35 µL of K18 Detection Antibody from the K18 Detection vial. Vortex the mixture for 30 seconds.

Once the mix is prepared it must be used within 2-3 minutes.

J) LiverAce™ COMBO Mix B

The LiverAce™ COMBO Mix B 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.

K) 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™ COMBO Kit 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 K18 Beads and add 25 µL to each well of the first 96-well plate.
2. Add 25 µL of each K18 Standard (Standard 1 to Standard 8) into the standard wells.
3. Add 25 µL of Serum Sample to the sample wells.
4. Seal and cover the plate and incubate with agitation on a plate shaker for 30 minutes at 30 ºC.
5. Place the first 96-well plate on the magnet for 1 minute, then carefully transfer the supernatants to the wells of the second 96-well plate.
6. Resuspend the remaining beads from the first 96-well plate in 200 µL of Wash Buffer 1X.

7. Vortex the diluted miR-122 Beads and add 25 µL to each well containing the supernatant on the second 96-well plate.

8. Add 25 µL of each miR-122 Standard (Standard 1 to Standard 8) into the standard wells.

9. Seal and cover the second 96-well plate and incubate with agitation on a plate shaker for 90 minutes at 30 ºC.

10. While the second 96-well plate is incubating, wash the first 96-well plate twice according to the instructions in the Plate Washing section.

11. Add 50 µL of LiverAce™ COMBO Mix A into each well of the first 96-well plate.

12. Seal and cover the plate and incubate with agitation on a plate shaker for 30 minutes at 30 ºC.

13. At the end of the incubation, add 100 µL of Wash Buffer 1X to each well of the first 96-well plate.

14. Place the first 96-well plate on the magnet for 1 minute, then gently remove well contents.

15. Resuspend the beads from the first plate in 100 µL of Wash Buffer 1X and store the plate at 2-8 ºC until the 'step 22' below (previously bring the plate to room temperature).

16. At the end of the incubation of the second 96-well plate, add 100 µL of Wash Buffer 1X to each well and wash it 3 times according to the instructions given in the Plate Wash section.

17. Add 50 µL of LiverAce™ COMBO Mix B into each well.

18. Seal and cover the plate and incubate with agitation on a plate shaker for 1 hour at 40 ºC.

19. At the end of the incubation, add 100 µL of Wash Buffer 1X to each well of the second 96-well plate.

20. Place the second 96-well plate on the magnet for 1 minute, then gently remove well contents.

21. Resuspend the beads from the second 96-well plate in 100 µL of Wash Buffer 1X.

22. Transfer the 100 µL from the wells containing Protein Beads from the first 96-well plate to the appropriate wells containing the 100 µL of miR-122 Beads on the second 96-well plate. Pipette up and down to thoroughly resuspend the Protein Beads, ensuring an efficient transfer.

23. Wash the plate twice following the instructions listed in the Plate Washing section.

24. Add 50 µL SA-PE to each well.

25. Seal and cover the plate and incubate with agitation on a plate shaker for 30 minutes at 30 ºC.

26. At the end of the incubation, add 100 µL of Wash Buffer 1X to each well and wash plate 3 times following the instructions listed in the Plate Washing section.

27. Add 120 µL of Wash Buffer to all wells. Resuspend the beads on a plate shaker for 5 minutes at 30 ºC.

28. Run plate on Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® instrument with xPONENT® software or xMAP® INTELLIFLEX instrument with INTELLIFLEX software.

LiverAce™ COMBO Kit Workflow

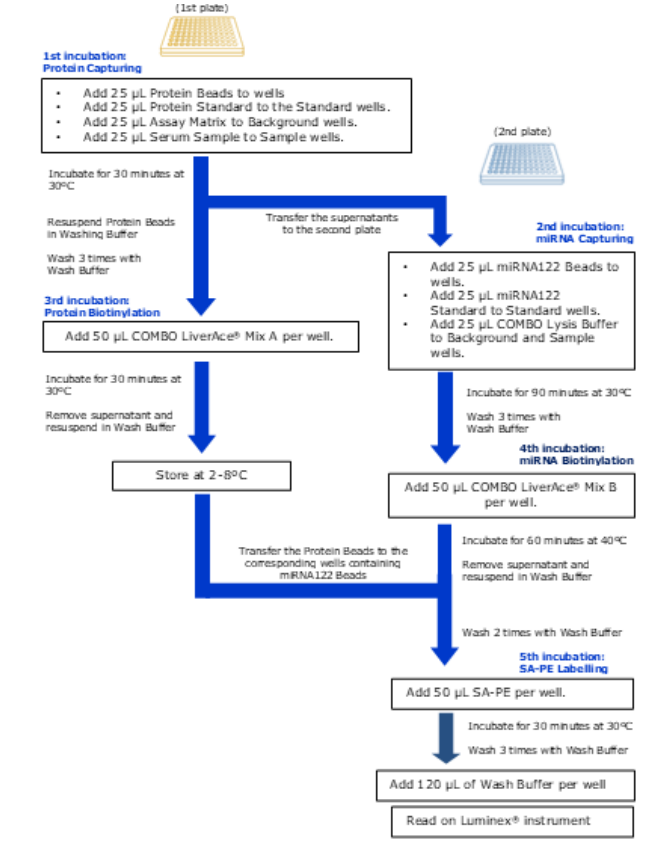


Plate Washing

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.

Place the 96-well plate on the magnet for 60 seconds to allow complete settling of the magnetic beads. Remove the well contents by gently decanting the plate into an appropriate waste receptacle and gently tapping it on absorbent pads to remove residual liquid. Wash plate with Wash Buffer 1X by removing plate from magnet, adding 200 µL Wash Buffer to each well, shaking for 30 seconds, reattaching plate to magnet, letting beads settle for 60 seconds and removing well contents as previously described. Repeat wash steps the number of times recommended in Assay Procedure.

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™ LABScam™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	Multi-plex Beads

K18	18
miR-122	19

Assay Characteristics

Cross-Reactivity

There was no or negligible cross reactivity between the miR-122 and K18 Beads 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)
K18	60	137
miR-122	2.4	20.58

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
K18	< 15%	< 25%
miR-122	< 15%	< 25%

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
K18	95-105%
miR-122	95-105%

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	0 pg/mL Standard (Background)	0 pg/mL Standard (Background)	0 pg/mL Standard (Background)	Sample 8	Sample 8	Sample 8	Sample 16	Sample 16	Sample 16	Sample 24	Sample 24	Sample 24

Destina Genomica S.L.
Avenida de la Innovación, 1
18006 Granada, Spain