mmu-miR-7052-5p miREIA

microRNA enzyme immunoassay kit

Product Data Sheet

Cat. No.: RDM0026M

For Research Use Only

BioVendor
Research
and Diagnostic Products

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This kit is manufactured by:

BioVendor – Laboratorní medicína a.s.

Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The RDM0026M mmu-miR-7052-5p miREIA is an enzyme immunoassay for the quantitative measurement of mouse microRNA-7052-5p.

FEATURES

- It is intended for research use only
- The total assay time is less than 2.5 hours
- The kit measures mmu-miR-7052-5p isolated from mouse blood, heparin plasma and tissue homogenates
- Assay format is 96 wells
- Quality Control is synthetic miRNA-based
- Standard is synthetic miRNA-based
- Components of the kit are provided ready to use, concentrated or dried

2. STORAGE AND EXPIRATION

Store the complete kit at 2–8 °C. Under these conditions, all components are stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 11.

3. INTRODUCTION

MicroRNAs (miRNAs) are small non-coding RNA molecules, approximately 22 nucleotides in length that regulate gene translation through silencing or degradation of target mRNAs. They are involved in multiple biological processes, including differentiation and proliferation, metabolism, hemostasis, apoptosis or inflammation, and in pathophysiology of many diseases. Numerous studies have suggested circulating miRNAs as promising diagnostic and prognostic biomarkers of many diseases.

4. TEST PRINCIPLE

BioVendor mmu-miR-7052-5p miREIA is an enzyme immunoassay for miRNA quantification which involves hybridization of miRNA isolated from a mouse sample to complementary biotinylated DNA probe for mmu-miR-7052-5p. The DNA/RNA hybrids are then transferred into microplate wells pre-coated with monoclonal antibody specific to perfectly matched DNA/RNA hybrids. After washing, the solid phase is incubated with streptavidin-HRP conjugate and after another washing step, the resulting complexes are visualized by chromogenic substrate. The absorbance is proportional to the concentration of mmu-miR-7052-5p.
A standard curve is constructed by plotting absorbance values against concentrations of mmu-miR-7052-5p standards. Concentrations of unknown samples and Quality Control are determined using this standard curve.

5. PRECAUTIONS

- **For professional use only**
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Stop and Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution, wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- **RNases are stable and robust enzymes that catalyze degradation of RNA. It is therefore indispensable to create an RNAse-free environment following the rules summarized below:**
  - The RNase-free working environment should be located away from microbiological work station
  - Use disposable gloves when handling reagents, samples, pipettes, and tubes
  - The gloves should be changed frequently to avoid contamination
  - Tips, tubes, lab coats, pipettes, etc. should be allocated for RNA work only
  - Nuclease-free water should be used
  - Commercial RNase decontamination solution should be used to clean all surfaces
  - Isolated RNA samples should be kept on ice
  - Use filter pipette tips
  - Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- Avoid any contamination among samples and reagents. For this purpose, disposable tips must be used for each sample and reagent. It is also recommended to establish and maintain separate areas for RNA isolation and miREIA detection
- Substrate Solution should remain colourless until added to the plate. Keep the Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENTS SUPPLIED

Kit Components	*State*	*Quantity*
DNA Probe Conc. (50x)	concentrated	0.1 ml
Master Standard	dried	2 vials
Quality Control	dried	2 vials
Antibody Coated Microtiter Strips	ready to use	96 wells
Streptavidin-HRP Conjugate	ready to use	13 ml
RNase Inhibitor Conc. (500x)	concentrated	0.05 ml
Dilution Buffer	ready to use	13 ml
Substrate Solution	ready to use	13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Commercially available RNase decontamination solution, e.g. RNaseZAP
- Disposable gloves
- Deionized (distilled) water
- Nuclease-free water (molecular biology grade nuclease-free water)
- Test tubes for diluting samples (nuclease-free PCR tubes)
- Nuclease-free, low nucleic acid binding tubes (1.5 ml)
- Glassware (graduated cylinder and bottle) for Wash Solution and RNase Inhibitor Solution
- Precision pipettes to deliver 5–1000 µl with disposable filter pipette tips (nuclease-free)
- Microplate sealing film or cover
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Centrifuge for < 2 ml tubes
- Thermoblock or thermal cycler
- Incubator for incubation at 37 °C
- Microplate washer (optional). [Manual washing is possible but not recommended.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550–650 nm)
- Software package facilitating data generation and analysis (optional)

9. PRE-ANALYTICAL PHASE

Sample Type

mmu-miR-7052-5p miREIA is validated for miRNA isolated from mouse whole blood, heparin plasma and tissue homogenates.

Ask for information at info@biovendor.com if assaying miRNA isolated from serum, plasma or another material.

Processing of whole blood

Conditions during sample collection may affect the detection of microRNAs. Therefore, it is highly recommended to follow standardized procedure for blood collection:

- To minimize patient variables, it is recommended to ensure overnight fasting prior to blood collection. Circadian rhythm, activity and diet are known to influence the microRNA levels
- Standardized needles and blood collection tubes are needed
- Gloves must be worn all the time when handling specimens
- Heparin Blood Collection Tubes are recommended for whole blood collection and storage. Follow the instructions for blood collection and handling provided by the manufacturer.
- Avoid repeated freeze-thaw cycles

10. RNA EXTRACTION

Samples can be assayed immediately after collection, or should be stored at -80 °C. It is necessary to isolate RNA before measuring by miREIA.
The users are supposed to choose an appropriate kit for RNA isolation themselves, depending on the sample type. For RNA isolation, use e.g. BioVendor RNA Isolation Kit (Cat. No.: RIK001, Cat. No.: RIK002).

Stability and Storage of RNA Samples

- RNA samples should be stored in nuclease-free plastic tubes. To avoid freeze-thaw cycles, divide the isolated RNA samples into aliquots
- When working with isolated RNA samples, keep them on ice
- Isolated RNA samples must be stored at -80 °C for long term storage

RNA Handling

- Wear gloves all the time when handling specimens and reagents
- Use RNase-free filter tips and tubes
- Create and maintain RNase-free working environment (specified in Chapter 6. Technical hints)

Spike-In Quality Control

It is recommended to normalize measured concentrations of mmu-miR-7052-5p by exogenous control. The concentration of miRNA measured by miREIA can be affected by efficiency of RNA isolation. For monitoring the efficiency of isolation, it is recommended to add a defined amount of synthetic nonhuman RNA Spike-In Control to the lysis buffer prior to starting the RNA isolation e.g. cel-miR-39-3p.
The concentration of Spike-In Control in samples is then measured by cel-miR-39-3p miREIA (Cat. No.: RDM0000C) using the DNA probe for Spike-In Control in parallel with the concentration of mmu-miR-7052-5p. To calculate the coefficient of isolation efficiency, the defined amount added to the samples prior to isolation is divided by the concentration of Spike-In Control measured by miREIA.
Finally, concentration of mmu-miR-7052-5p measured by miREIA should be multiplied by the coefficient of isolation efficiency for every sample.

11. PREPARATION OF REAGENTS

- **All reagents need to be brought to room temperature prior to use**
- **Always prepare only the appropriate quantity of reagents for your test**
- **Do not use components after the expiration date marked on their label**

ASSAY REAGENTS SUPPLIED READY TO USE

Antibody Coated Microtiter Strips

Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2–8 °C and protected from the moisture.

Streptavidin-HRP Conjugate
Dilution Buffer
Substrate Solution
Stop Solution

Stability and storage:
Opened reagents are stable 3 months when stored at 2–8 °C.

ASSAY REAGENTS SUPPLIED CONCENTRATED OR DRIED

RNase Inhibitor Conc. (500x)

Dilute RNase Inhibitor Conc. (500x) with nuclease-free water (not included in the kit) 500-fold, e.g. 10 µl of RNase Inhibitor Concentrate (500x) + 4 990 µl of nuclease-free water to obtain **RNase Inhibitor working solution.**
Stability and storage:
Opened RNase Inhibitor Conc. (500x) is stable until the expiration date when stored at 2–8 °C. Protect the RNase Inhibitor Conc. (500x) from light. Do not freeze the RNase Inhibitor Conc. (500x).
Do not store the diluted RNase Inhibitor solution.

Master Standard

Refer to the Certificate of Analysis for current volume of the RNase Inhibitor working solution needed for reconstitution of Master Standard!!!
Reconstitute the dried Master Standard with RNase Inhibitor working solution just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Vortex is recommended. The resulting concentration of mmu-miR-7052-5p in the stock solution is **25 amol/µl**.

Prepare set of standards using the RNase Inhibitor working solution as follows:

Volume of Standard	*Volume of RNase Inhibitor working solution*	*Concentration*
mmu-miR-7052-5p stock	-	25.0 amol/µl
50 µl of mmu-miR-7052-5p stock	50 µl	12.5 amol/µl
50 µl of 12.5 amol/µl	50 µl	6.25 amol/µl
50 µl of 6.25 amol/µl	50 µl	3.13 amol/µl
50 µl of 3.13 amol/µl	50 µl	1.56 amol/µl
50 µl of 1.56 amol/µl	50 µl	0.78 amol/µl
50 µl of 0.78 amol/µl	50 µl	0.39 amol/µl

Prepared set of standards are ready for hybridization.
Stability and storage:
Do not store the reconstituted and/or diluted set of standards.

DNA Probe Conc. (50x)

Dilute the DNA Probe Conc. (50x) fifty-fold in Dilution Buffer. Example: for 1 strip (8 wells) 5 µl of DNA Probe Conc. (50x) + 245 µl Dilution Buffer.
The DNA Probe working solution is to be used for hybridization with standards, Quality Control, RNA samples and blank.
Stability and storage:
Opened DNA Probe Conc. (50x) is stable 3 months when stored at 2–8 °C.
Do not store the diluted DNA Probe solution.

Quality Control

Refer to the Certificate of Analysis for current Quality Control concentration!!!
Reconstitute the Quality Control with 50 µl of RNase Inhibitor working solution just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Vortex is recommended.
Dilute reconstituted Quality Control 10x with RNase Inhibitor working solution, e.g. 5 µl of Quality Control + 45 µl of RNase Inhibitor working solution.
Diluted Quality Control is to be used for hybridization with the DNA Probe working solution.
Stability and storage:
Do not store the reconstituted and/or diluted Quality Control.

Note: Concentration of miRNA in Quality Control need not be anyhow associated with normal and/or pathological concentrations in samples. Quality Control serves just for control that the kit works in accordance with PDS and CoA and that miREIA test was carried out properly.

Wash Solution Conc. (10x)

Dilute Wash Solution Conc. (10x) ten-fold in distilled water to obtain **diluted Wash Solution**. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.
Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2–8 °C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2–8 °C.

12. PREPARATION OF SAMPLES

An appropriate dilution of isolated RNA samples should be assessed by the researcher prior to batch measurement.

Recommended starting dilution for RNA isolated from whole blood is 40x.
Dilute RNA samples **40x** with RNase Inhibitor working solution just prior to the assay, e.g. 3 µl of sample + 117 µl RNAase Inhibitor working solution. **Mix well** (not to foam). Vortex is recommended.
Do not store the diluted samples.

Recommended starting dilution for RNA isolated from tissue homogenates is 1 200x. Dilute RNA samples just prior to the assay in two steps as follows:
Dilution A (20x):
Add 3µl of sample into 57 µl of RNase Inhibitor working solution. **Mix well** (not to foam). Vortex is recommended.
Dilution B (60x):
Add 5 µl of Dilution A into 285 µl of RNase Inhibitor working solution to prepare final dilution (1 000x). **Mix well** (not to foam). Vortex is recommended.
Do not store the diluted samples.

Note: It is recommended to use a precise pipette and a careful technique to perform the dilution in order to get precise results.

13. ASSAY PROCEDURE

PREPARATION OF HYBRIDS

The procedure described below must be performed for each point of the standard curve, Quality Control, sample and blank separately (see the Hybridization Procedure Summary below).

1. Pipet **20 µl** of DNA Probe working solution into each nuclease-free PCR tube.
2. Add **20 µl** of prepared standards, RNase Inhibitor working solution (=Blank), Quality Control or diluted RNA samples, respectively - refer to the tables below.

Prepare hybrids for standard curve as follows:

Volume of DNA Probe solution	*Volume of set of standards*
20 µl	20 µl of 25.0 amol/µl
20 µl	20 µl of 12.5 amol/µl
20 µl	20 µl of 6.25 amol/µl
20 µl	20 µl of 3.13 amol/µl
20 µl	20 µl of 1.56 amol/µl
20 µl	20 µl of 0.78 amol/µl
20 µl	20 µl of 0.39 amol/µl

Prepare hybrids for blank as follows:

Volume of DNA Probe solution	*Volume of RNase Inhibitor working solution*
20 µl	20 µl

Prepare hybrids for isolated RNA samples as follows:

Volume of DNA Probe solution	*Volume of diluted sample*
20 µl	20 µl

Prepare hybrids for Quality Control as follows:

Volume of DNA Probe solution	*Volume of diluted Quality Control*
20 µl	20 µl

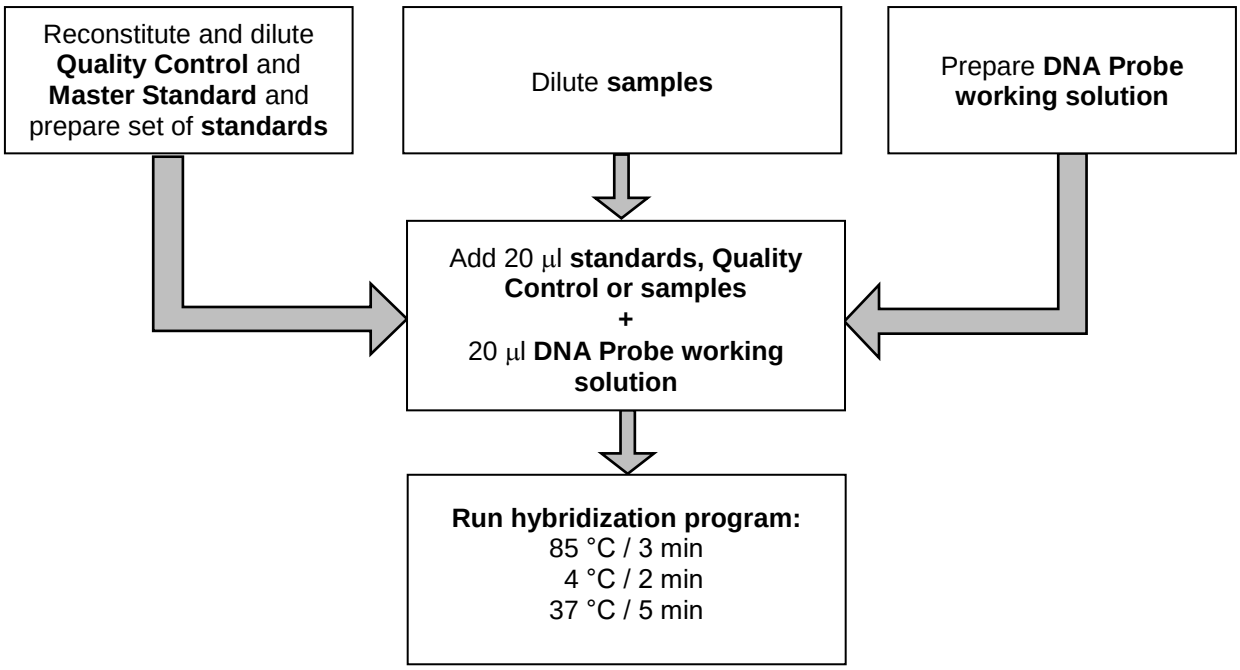
HYBRIDIZATION PROCEDURE

Insert nuclease-free tubes with the hybrids prepared in the previous step into a cycler and run the hybridization program. It is also possible to use a thermoblock instead of the cycler.

Hybridization program:

1. 85 °C / 3 min
2. 4 °C / 2 min
3. 37 °C / 5 min

Hybridization Procedure Summary



miREIA PROCEDURE

1. Dilute prepared hybrids 3-fold with the Dilution Buffer (in tubes). Example: 35 µl of hybrid + 70 µl of Dilution Buffer. Mix gently (not to foam).
2. Pipet **90 µl** of diluted hybrids into the appropriate wells of the microtiter plate (see *Figure 1)*. Covering the plate with e.g. microplate cover or sealing film is recommended.
3. Incubate the plate at 37 °C for **1 hour**, without shaking.
4. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
5. Add **100 µl** of Streptavidin-HRP Conjugate into each well. Covering the plate with e.g. microplate cover or sealing film is recommended.
6. Incubate the plate at 37 °C for **30 minutes**, without shaking.
7. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
8. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
9. Incubate the plate for **15 minutes** at room temperature. The incubation time may be extended [up to 30 minutes] if the reaction temperature is below 20 °C. Do not shake the plate during the incubation.
10. Stop the colour development by adding **100 µl** of Stop Solution into each well.
11. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550–650 nm). Subtract readings at 630 nm (550–650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 10.**

Note 1: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine hsa-miR-145-5p concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing 5-times: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat four times. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1	strip 2	strip 3	strip 4	strip 5	strip 6	strip 7	strip 8	strip 9	strip 10	strip 11	strip 12
A	**Std. 25.0**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	Sample 48	Sample 56	Sample 64	Sample 72	Sample 80
B	**Std. 12.5**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41	Sample 49	Sample 57	Sample 65	Sample 73	Sample 81
C	**Std. 6.25**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34	Sample 42	Sample 50	Sample 58	Sample 66	Sample 74	Sample 82
D	**Std. 3.13**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35	Sample 43	Sample 51	Sample 59	Sample 67	Sample 75	Sample 83
E	**Std. 1.56**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36	Sample 44	Sample 52	Sample 60	Sample 68	Sample 76	Sample 84
F	**Std. 0.78**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37	Sample 45	Sample 53	Sample 61	Sample 69	Sample 77	Sample 85
G	**Std. 0.39**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38	Sample 46	Sample 54	Sample 62	Sample 70	Sample 78	Sample 86
H	**Quality Control**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	Sample 47	Sample 55	Sample 63	Sample 71	Sample 79	Sample 87

Figure 1: Example of a work sheet.

14. CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration.
The Standard curve is constructed by plotting the mean absorbance of Standards (Y) against the known concentration of Standards (X) in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of mmu-miR-7052-5p (amol/µl) in samples.

The measured concentration of samples and Quality Control calculated from the standard curve must be multiplied by their respective dilution factor, because samples and Quality Control have been diluted prior to the assay, e.g. 0.48 amol/µl (from standard curve) x 40 (dilution factor) = 19.2 amol/µl.

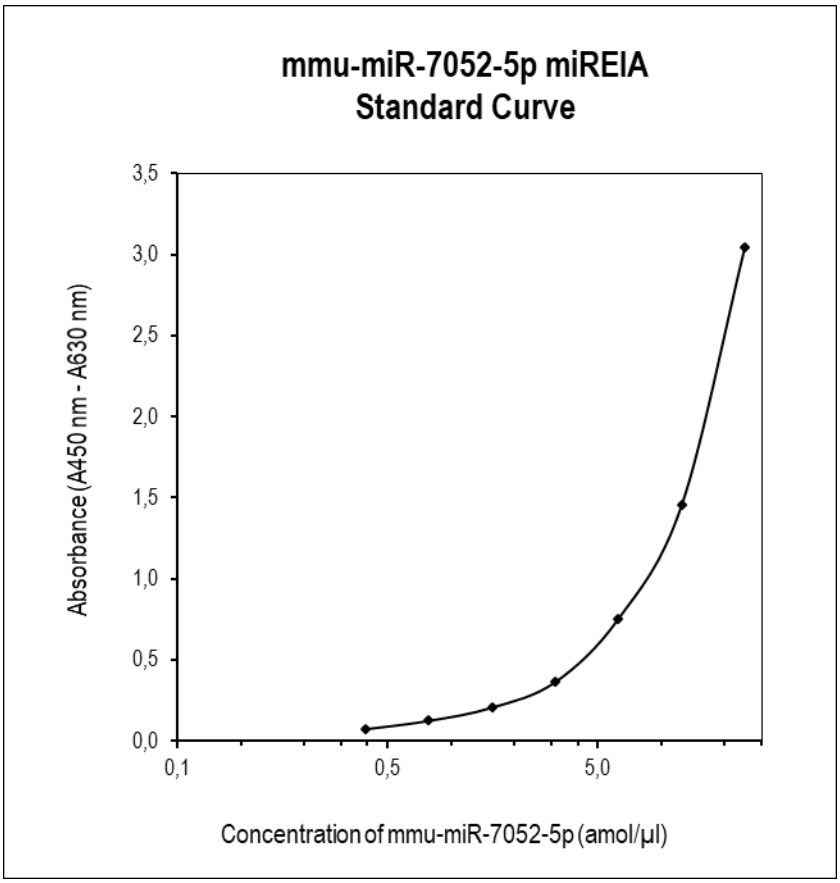


Figure 2: Typical standard curve for mmu-miR-7052-5p miREIA.

15. PERFORMANCE CHARACTERISTICS

- **Typical analytical data of BioVendor mmu-miR-7052-5p miREIA are presented in this chapter.**

Sensitivity

Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank plus three standard deviations of the absorbance of blank: A_{blank} + 3xSD_{blank} is calculated from the real mmu-miR-7052-5p values in wells and is 0.013 amol/µl.

Limit of Assay

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

Specificity

The DNA Probe for mmu-miR-7052-5p is complementary to the sequence of mmu-miR-7052-5p.

- **Presented results are multiplied by respective dilution factor**

Precision

Intra-assay (Within-Run) (n=8)

Sample	*Mean (amol/µl)*	*SD (amol/µl)*	*CV (%)*
1	7.14	0.55	7.7
2	3.80	0.19	5.1

Inter-assay (Run-to-Run) (n=5)

Sample	*Mean (amol/µl)*	*SD (amol/µl)*	*CV (%)*
1	32.79	3.88	11.8
2	77.03	6.76	8.8

Spiking Recovery

Isolated RNA samples were diluted, spiked with different amounts of mmu-miR-7052-5p and assayed.

Sample	***O**bserved (amol/µl)*	***E**xpected (amol/µl)*	*Recovery **O/E** (%)*
1	834.3	-	-
	2786.5	2834.3	98.3
	1723.2	1834.3	93.9
	1283.5	1333.5	96.2
2	544.8	-	-
	1659.6	1544.8	107.4
	115.2	1044.8	106.7
	786.1	794.4	98.9

Linearity

Isolated RNA samples were serially diluted and assayed.

Sample	*Dilution*	***O**bserved (amol/µl)*	***E**xpected (amol/µl)*	*Recovery **O/E** (%)*
1	-	634.7	-	-
	2x	336.5	317.3	106.0
	4x	180.9	158.7	114.0
	8x	88.38	79.33	114.4
2	-	565.4	-	-
	2x	299.2	282.7	105.9
	4x	131.9	141.3	93.3
	8x	78.30	70.67	110.8

Reference range

It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for mmu-miR-7052-5p levels with the assay.

16. DEFINITION OF THE STANDARD

The synthetic mmu-miR-7052-5p is used as the standard for mmu-miR-7052-5p quantification.

17. TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Control or samples

Degraded RNA

In most cases degradation of RNA is caused by RNases. Keep RNase free environment when working with RNA (see Chapter 6)

18. REFERENCES

References to mmu-miR-7052-5p:

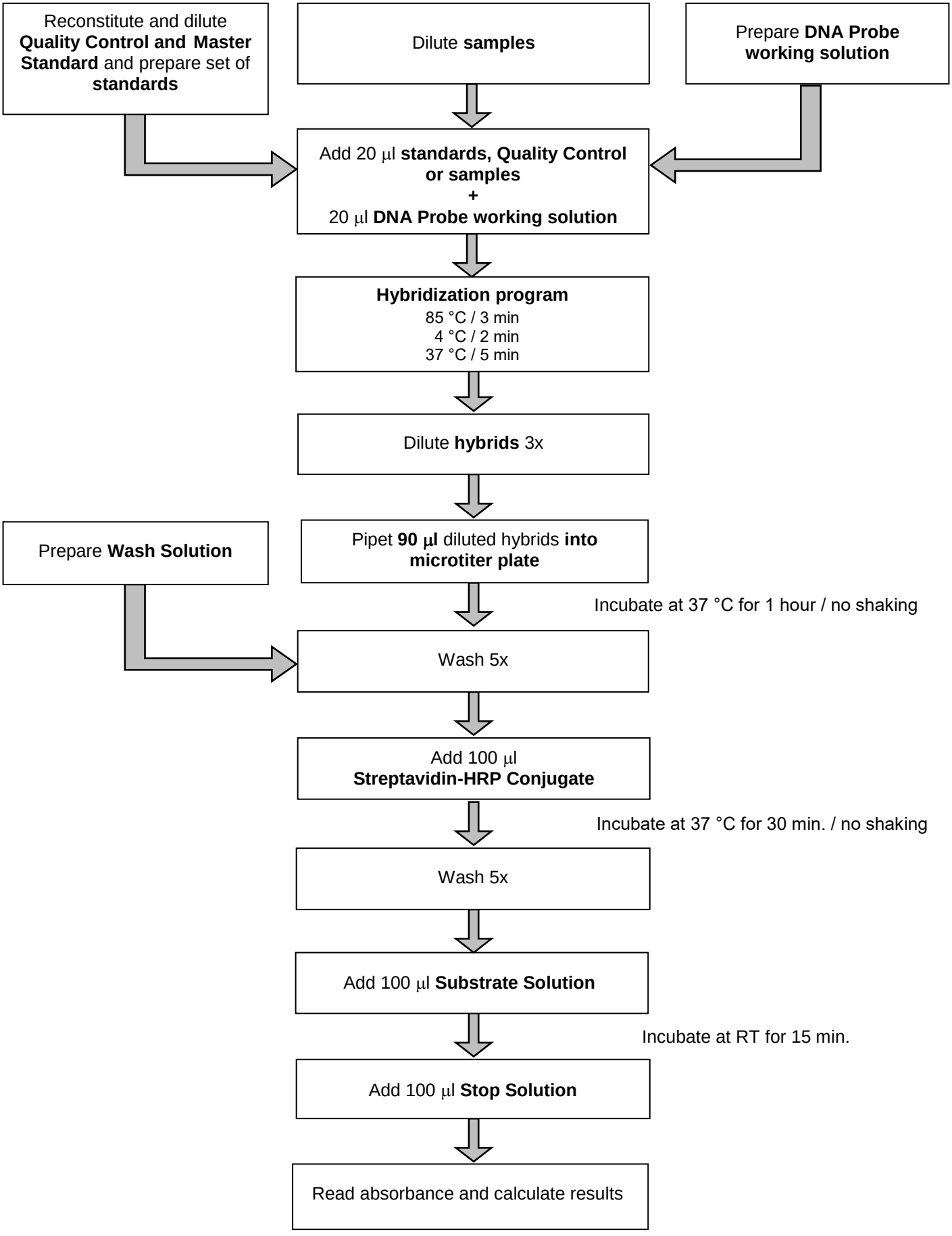
1. Ladewig E, Okamura K, Flynt AS, Westholm JO and Lai EC: Discovery of hundreds of mirtrons in mouse and human small RNA data. Published by Cold Spring Harbor Laboratory Press; 22:1634–1645 (2012).
2. Wang Y, Wei ChX, Shao LQ and Zhao M: MiRNA Signaling in Viral Myocarditis Novel and Unique Pathological Features. Acta Cardiol Sin 34:77-86 (2018)

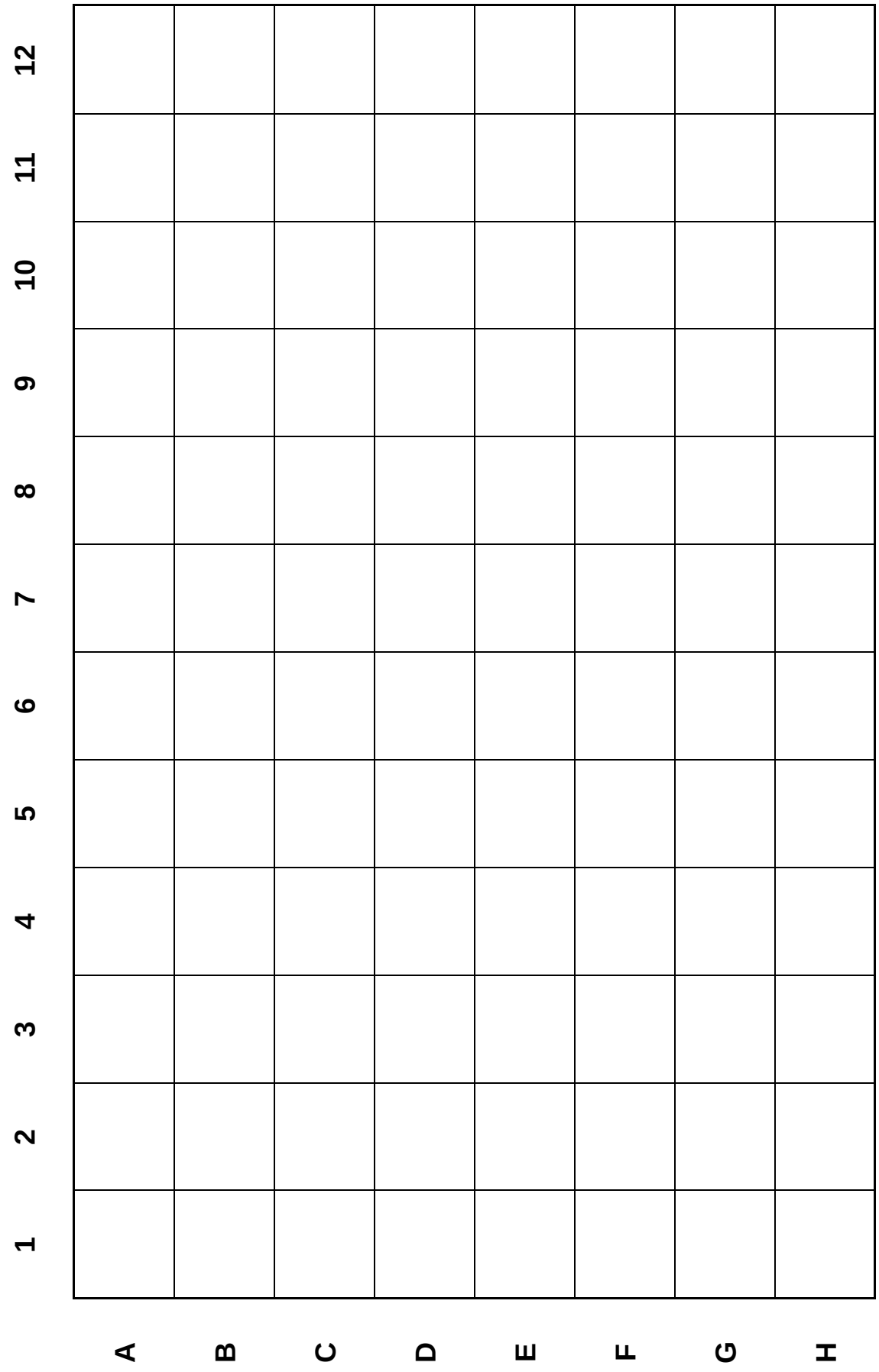
- **For more references on this product see our WebPages at www.biovendor.com**

19. EXPLANATION OF THE SYMBOLS

	Catalogue number
	Content
	Lot number
	Attention, see instructions for use
	Expiry date
	Storage conditions
	Name and registered office of the manufacturer

Assay Procedure - summary

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20. NOTES

There are BioVendor branches and distributors near you.
To find the office closest to you, visit **www.biovendor.com/contact**

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