



cel-miR-39-3p miREIA

microRNA enzyme immunoassay kit

Spike-In Control miREIA

Product Data Sheet

Cat. No.: RDM0000C

For Research Use Only





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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 RDM0000C cel-miR-39-3p miREIA is an enzyme immunoassay for the quantitative measurement of *Caenorhabditis elegans* microRNA-39-3p.

FEATURES

- It is intended for research use only
- The total assay time is less than 2.5 hours
- The kit measures concentration of exogenous cel-miR-39-3p spiked in RNA isolates
- Assay format is 96 wells
- 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 10.



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 the pathophysiology of many diseases. Numerous studies have suggested circulating miRNAs as promising diagnostic and prognostic biomarkers of many diseases.

Concentration of target miRNA measured by miREIA can be affected by efficiency of RNA isolation. Therefore, it is recommended to normalize the measured concentration of target miRNA to the efficiency of RNA isolation using an exogenous control.

Efficiency of RNA isolation can be monitored by adding a defined amount of synthetic non-human miRNA, e.g. cel-miR-39-3p miRNA, to the sample during RNA isolation. The synthetic RNA is processed the same way as the target RNA present in the samples.

After RNA isolation, concentration of exogenous cel-miR-39-3p added to the samples is measured by cel-miR-39-3p miREIA in parallel with the concentration of the target miRNA. To calculate the coefficient of isolation efficiency, the amount of cel-miR-39-3p added to the samples prior to isolation is divided by the concentration of cel-miR-39-3p measured by miREIA.

Finally, the concentration of target miRNA measured by miREIA is multiplied by the coefficient of isolation efficiency for every sample.

If such a normalization is applied, then, each RNA isolate sample is measured by two miREIA kits: one kit for determination of the target miRNA (samples are diluted as recommended in the corresponding datasheet) and by a second kit, cel-miR-39-3p miREIA (samples are diluted 10-fold as written in page 15).



4. TEST PRINCIPLE

BioVendor cel-miR-39-3p miREIA is an immunoassay for quantification of cel-miR-39-3p used as an exogenous spike-in control. The assay involves hybridization of cel-miR-39-3p to complementary biotinylated DNA probe. The DNA/miRNA hybrids are then transferred into microplate wells pre-coated with monoclonal antibody specific to perfectly matched DNA/miRNA hybrids. After washing, the solid phase is incubated with streptavidin-HRP conjugate and after another washing step, the resulting complexes are visualized using a chromogenic substrate. The absorbance is proportional to the concentration of cel-miR-39-3p.

A standard curve is constructed by plotting absorbance values against concentrations of cel-miR-39-3p standards. Concentration of cel-miR-39-3p in samples is 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

<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
DNA Probe Conc. (50x)	concentrated	0.1 ml
Master Standard	dried	2 vials
Spike-In 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
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 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 μ l 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 \pm 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. RNA EXTRACTION AND SPIKING

Prior to miREIA analysis, it is necessary to isolate RNA from the samples.

The users should 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).

To normalize miRNA concentrations measured by miREIA to the efficiency of RNA isolation, synthetic RNA Spike-In Control is added to the samples during RNA isolation. See chapter 11. Spiking Procedure.

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)



10. 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 cel-miR-39-3p in the stock solution is **12.5 amol/μl**.

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

<i>Volume of Standard</i>	<i>Volume of RNase Inhibitor working solution</i>	<i>Concentration</i>
cel-miR-39-3p stock	-	12.5 amol/μl
50 μl of cel-miR-39-3p stock	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.

Spike-In Control

Refer to the Certificate of Analysis for current volume of the RNase Inhibitor working solution needed for reconstitution of Spike-In Control!

Reconstitute the dried Spike-In Control with RNase Inhibitor working solution just prior to RNA isolation. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Vortex is recommended. The resulting concentration of Spike-In control in the stock solution is 10 000 amol/μl.

Prepared Spike-In Control is ready to use

Stability and storage:

The Spike-In Control stock solution is stable 1 week when stored at 2–8 °C.

DNA Probe Conc. (50x)

Dilute the DNA Probe Conc. (50x) in Dilution Buffer fifty-fold. Example: for 1 strip (8 wells) 5 μ l of DNA Probe Conc. (50x) + 245 μ l Dilution Buffer.

The DNA Probe working solution is to be used for hybridization with standards, 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.

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.



11. SPIKING PROCEDURE

The Spike-In Control must not be added to the samples prior to RNA isolation since it would be degraded by RNases. Therefore, the Spike-In Control is added after addition of lysis/denaturing buffer.

1. Reconstitute the dried Spike-In Control with appropriate volume of RNase Inhibitor working solution – refer to chapter 10. Preparation of Reagents.
2. Calculate appropriate volume of Spike-In Control stock solution that is to be added to the samples based on the expected volume of RNA eluate. Recommended final concentration of cel-miR-39-3p spike in the samples is 1 000 amol/ μ l.
Example: If the expected volume of RNA isolate is 45 μ l, then, 5 μ l of Spike-In Control stock solution should be added to the sample during RNA isolation.
3. Add the calculated volume of Spike-In Control stock solution to the samples after addition of lysis buffer.

CALCULATION OF NORMALIZED CONCENTRATION OF THE TARGET MIRNA

Calculate the coefficient of isolation efficiency

To calculate isolation efficiency, concentration of cel-miR-39-3p spike added to the samples is divided by the concentration of cel-miR-39-3p measured by miREIA.

Example:

If the concentration of cel-miR-39-3p spike added to the samples is 1 000 amol/ μ l and the concentration of cel-miR-39-3p measured by miREIA is 100 amol/ μ l, then, the calculated coefficient of isolation efficiency is **10**.

Calculate the normalized concentration of target miRNA

The concentration of target miRNA measured by miREIA is multiplied by the coefficient of isolation efficiency.

Example:

If the concentration of target miRNA measured by miREIA is 150 amol/ μ l and the coefficient of isolation efficiency is 10, then, the normalized concentration of target miRNA is 1 500 amol/ μ l.



12. PREPARATION OF SAMPLES

Recommended starting dilution of isolated RNA samples spiked with the Spike-In Control is 10x

Dilute RNA samples **10x** with RNase Inhibitor working solution just prior to the assay, e.g. 5 μ l of sample + 45 μ l RNase Inhibitor working solution. **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, 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) or diluted RNA samples, respectively - refer to the tables below.

Prepare hybrids for standard curve as follows:

<i>Volume of DNA Probe solution</i>	<i>Volume of standards</i>
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:

<i>Volume of DNA Probe solution</i>	<i>Volume of RNase Inhibitor working solution</i>
20 µl	20 µl

Prepare hybrids for isolated RNA samples as follows:

<i>Volume of DNA Probe solution</i>	<i>Volume of diluted sample</i>
20 µl	20 µl

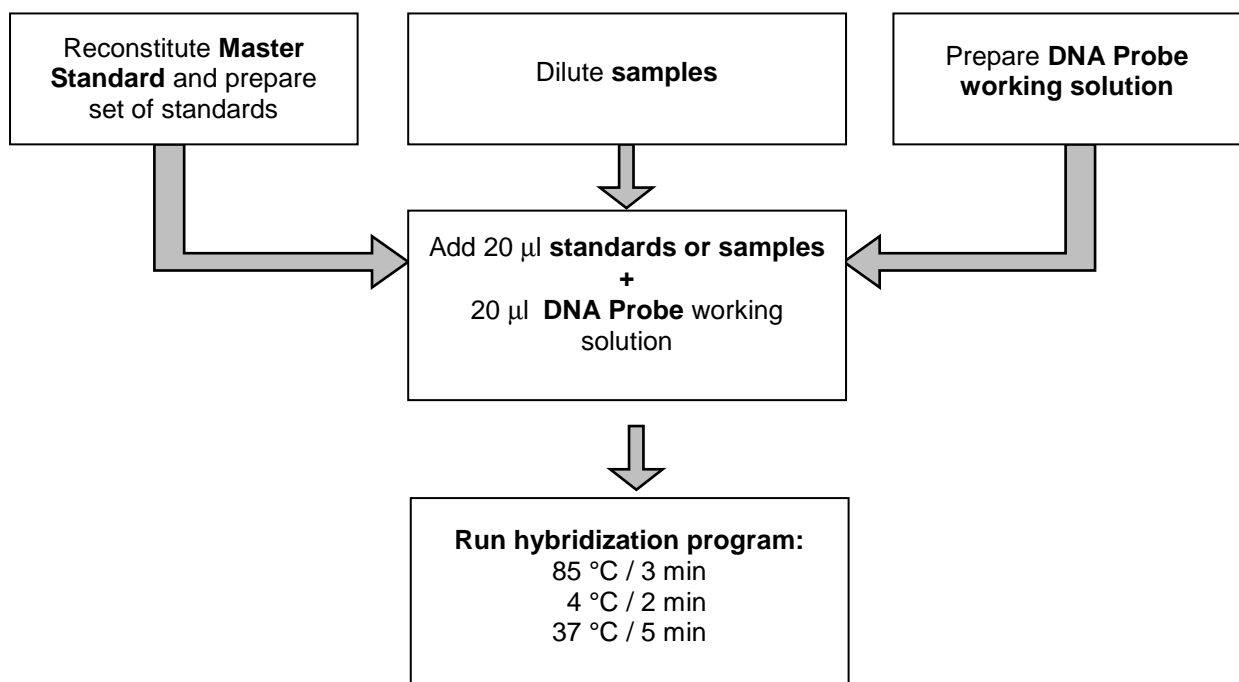
HYBRIDIZATION PROCEDURE

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 μ l of hybrid + 70 μ l of Dilution Buffer. Mix gently (not to foam).
2. Pipet **90 μ l** 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 μ l** 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 μ l** 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 μ l** of Stop Solution.
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 cel-miR-39-3p 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. 12.5	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41	Sample 49	Sample 57	Sample 65	Sample 73	Sample 81
B	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
C	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
D	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
E	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
F	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
G	Blank	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	Sample 47	Sample 55	Sample 63	Sample 71	Sample 79	Sample 87
H	Blank	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	Sample 48	Sample 56	Sample 64	Sample 72	Sample 80	Sample 88

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 cel-miR-39-3p (amol/ μ l) in samples.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 0.48 amol/ μ l (from standard curve) x 10 (dilution factor) = 4.8 amol/ μ l.

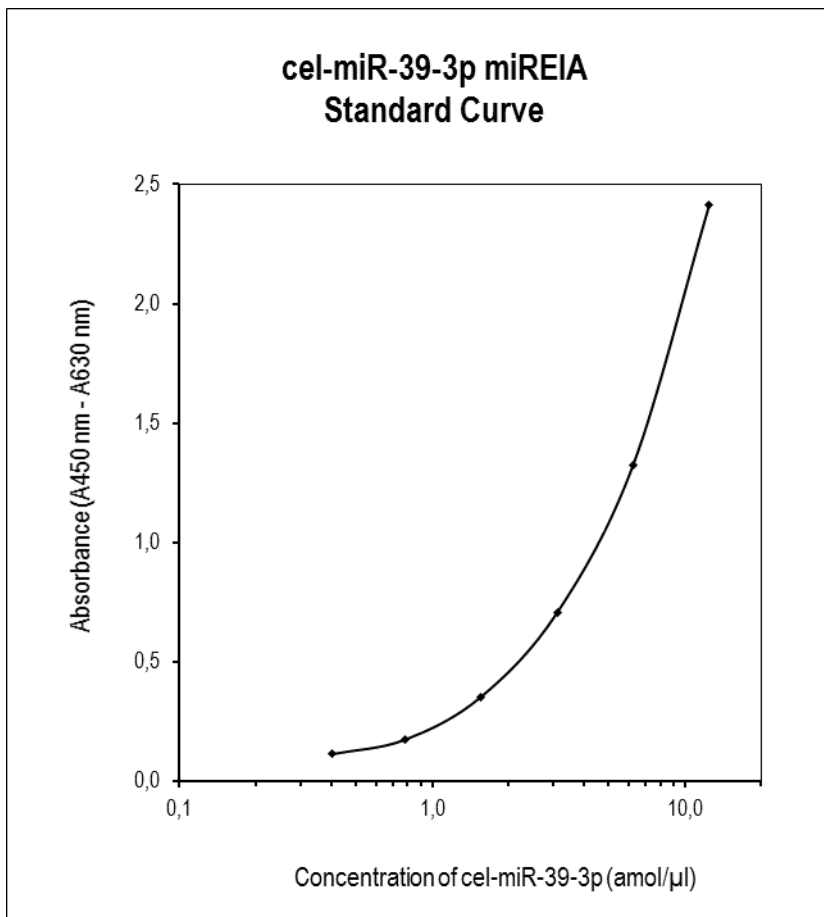


Figure 2: Typical standard curve for cel-miR-39-3p miREIA.



15. PERFORMANCE CHARACTERISTICS

- **Typical analytical data of BioVendor cel-miR-39-3p 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: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is calculated from the real cel-miR-39-3p values in wells and is 0.13 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 cel-miR-39-3p is complementary to the sequence of cel-miR-39-3p.

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

Precision

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

<i>Sample</i>	<i>Mean (amol/μl)</i>	<i>SD (amol/μl)</i>	<i>CV (%)</i>
1	3.40	0.19	5.6
2	1.53	0.17	10.9

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

<i>Sample</i>	<i>Mean (amol/μl)</i>	<i>SD (amol/μl)</i>	<i>CV (%)</i>
1	34.85	3.22	9.2
2	14.69	0.80	5.5

Spiking Recovery

Samples were spiked with different amounts of cel-miR-39-3p and assayed.

<i>Sample</i>	<i>Observed (amol/μl)</i>	<i>Expected (amol/μl)</i>	<i>Recovery O/E (%)</i>
1	119.60	-	-
	397.20	369.60	107.5
	283.84	244.40	116.1
	190.40	182.00	104.6
2	135.12	-	-
	404.64	385.12	105.1
	288.16	260.12	110.8
	205.60	197.52	104.1

Linearity

Samples spiked with cel-miR-39-3p were serially diluted and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (amol/μl)</i>	<i>Expected (amol/μl)</i>	<i>Recovery O/E (%)</i>
1	-	71.48	-	-
	2x	33.02	35.74	92.4
	4x	17.33	17.87	97.0
	8x	8.61	8.94	96.4
2	-	40.88	-	-
	2x	18.14	20.44	88.7
	4x	9.28	10.22	90.8
	8x	5.37	5.11	105.1



16. DEFINITION OF THE STANDARD

Synthetic cel-miR-39-3p is used as the standard for cel-miR-39-3p 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, cel-miR-39-3p or samples






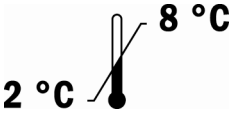

Degraded RNA

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

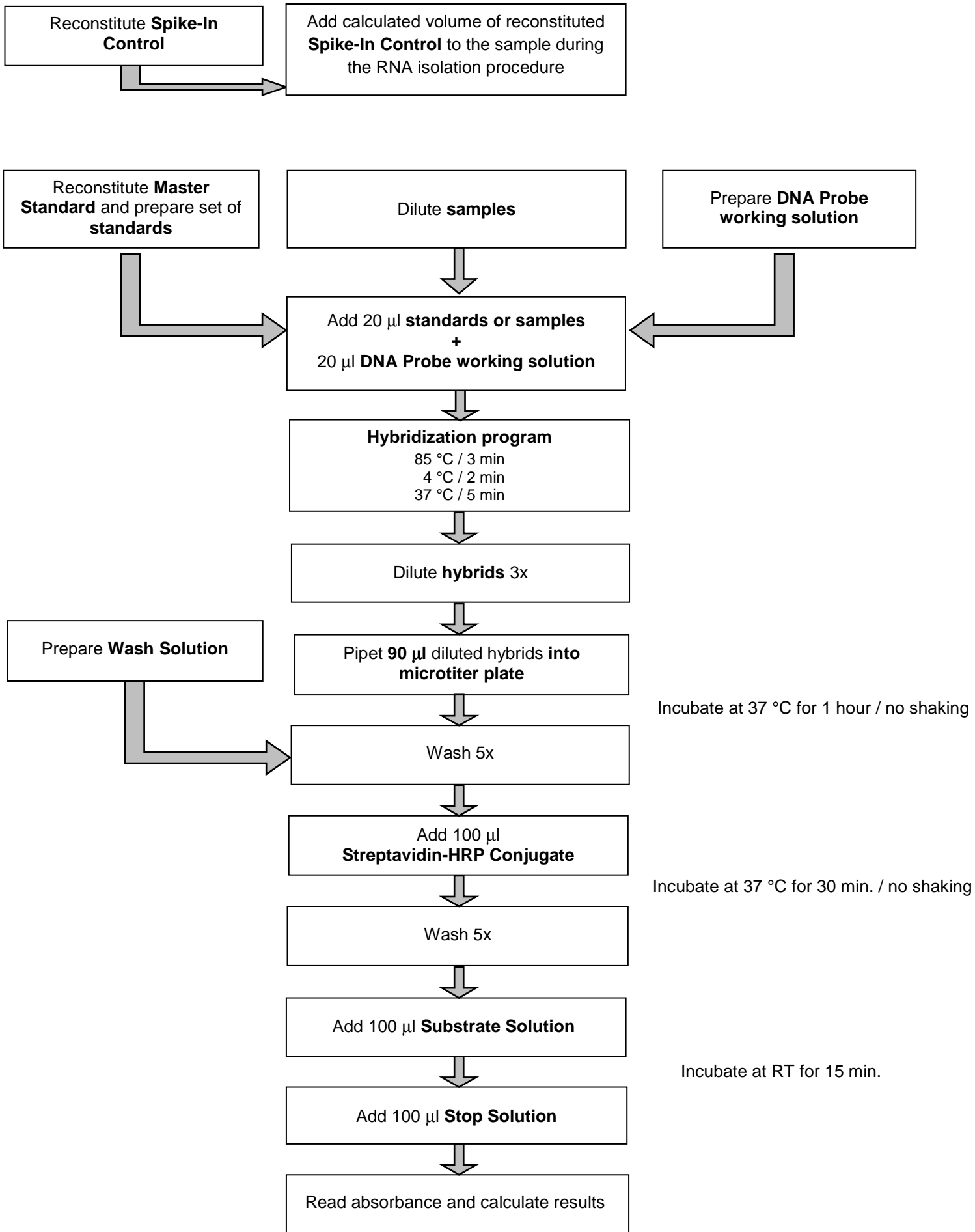
- **For more references on this product see our WebPages at www.biovendor.com**



18. 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



12
11
10
9
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6
5
4
3
2
1

A B C D E F G H



19. NOTES



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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