



## hsa-miR-126-3p miREIA

microRNA enzyme immunoassay kit

Product Data Sheet

**Cat. No.: RDM0018H**

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 RDM0018H hsa-miR-126-3p miREIA is an enzyme immunoassay for the quantitative measurement of human microRNA-126-3p.

### FEATURES

- It is intended for research use only
- The total assay time is less than 2.5 hours
- The kit measures hsa-miR-126-3p isolated from human blood and cell culture lysates
- 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 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.

The gene for miR-126 is located on chromosome 9. miR-126-3p is endothelial-specific miRNA that regulates angiogenesis and blood vessel integrity [1].

miR-126-3p also regulates expression of many cancer-related genes [2]. Studies suggest that miR-126-3p acts as either a tumor suppressor or an oncogene in different types of cancer [3, 4]. Lower expression of miR-126-3p has been reported to be associated with poor prognosis in hepatocellular carcinoma. Furthermore, overexpression of miR-126-3p significantly reduced tumor size [5]. The expression of miR-126-3p was also downregulated in malignant thyroid tumor samples when compared to benign thyroid tumor samples [6]. On the other hand, decreased miR-126-3p expression is associated with clinically more aggressive papillary thyroid cancer [2].

It was shown that levels of miR-126-3p in endothelial cell microparticles are abnormal in subjects with pre-diabetes and correlate with well-accepted markers of endothelial dysfunction [7]. It was demonstrated that downregulated plasma level of miR-126-3p in patients with type 2 diabetes is associated with diabetic vascular complications [8].

It was also found that miR-126-3p regulates lipid metabolism through targeting the genes related to lipid synthesis [9].

Several groups have revealed that miR-126-3p levels are low in serum of stable coronary artery disease patients, and that there is a positive association between circulating miR-126-3p and myocardial infarction [10, 11]. Significantly lower level of miR-126-3p expression was observed in severe coronary artery disease patients compared to the healthy subjects [12].

### **Areas of investigation**

Oncology



## 4. TEST PRINCIPLE

BioVendor hsa-miR-126-3p miREIA is an enzyme immunoassay for miRNA quantification which involves hybridization of miRNA isolated from a patient sample to complementary biotinylated DNA probe for hsa-miR-126-3p. The DNA/RNA 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 by chromogenic substrate. The absorbance is proportional to the concentration of hsa-miR-126-3p.

A standard curve is constructed by plotting absorbance values against concentrations of hsa-miR-126-3p standards. Concentrations of unknown samples 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

| <i>Kit Components</i>                        | <i>State</i> | <i>Quantity</i> |
|--|--------------|-----------------|
| DNA Probe Conc. (50x)                        | concentrated | 0.1 ml          |
| Master Standard                              | 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  $\mu\text{l}$  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. PRE-ANALYTICAL PHASE

### Sample Type

hsa-miR-126-3p miREIA is validated for miRNA isolated from human whole blood and cell culture lysates (HEK cell lines).

Ask for information at [info@biovendor.com](mailto:info@biovendor.com) if assaying miRNA isolated from serum or plasma.

### 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
- PAXgene Blood RNA Tubes are recommended for whole blood collection and storage. Follow the instructions for blood collection and handling provided by the manufacturer:  
<http://www.preanalytix.com/products/blood/RNA/paxgene-blood-rna-tube>
- Immediately after blood collection, gently invert the PAXgene RNA tubes 10 times, then let the tubes stand in upright position for at least 2 hours (max. 72 hours)

### Stability and Storage:

- Store the PAXgene Blood RNA Tubes (with samples) up to 3 days at room temperature (15–25 °C), up to 5 days at 2–8 °C, or up to 8 years at –20 °C or –70 °C
- Bring the PAXgene Blood RNA Tubes to room temperature (approximately 2 hours are necessary); do not increase the temperature above 25 °C. Carefully invert the tubes 10 times
- 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 miREA.

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 hsa-miR-126-3p 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 hsa-miR-126-3p. 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 hsa-miR-126-3p 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 hsa-miR-126-3p 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> |
|-------------------------------|---|----------------------|
| hsa-miR-126-3p stock          | -   | 12.5 amol/μl         |
| 50 μl of hsa-miR-126-3p 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.**

### **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, 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.



## 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 20x.**

Dilute RNA samples **20x** with RNase Inhibitor working solution just prior to the assay, e.g. 5 µl of sample + 95 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 cell culture lysates (HEK cell lines) is 5x.**

Dilute RNA samples **5x** with RNase Inhibitor working solution just prior to the assay, e.g. 6 µl of sample + 24 µl 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  $\mu$ l** of DNA Probe working solution into each nuclease-free PCR tube.
2. Add **20  $\mu$ l** 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 set of standards</i> |
|-------------------------------------|-----------------------------------|
| 20 $\mu$ l                          | 20 $\mu$ l of 12.5 amol/ $\mu$ l  |
| 20 $\mu$ l                          | 20 $\mu$ l of 6.25 amol/ $\mu$ l  |
| 20 $\mu$ l                          | 20 $\mu$ l of 3.13 amol/ $\mu$ l  |
| 20 $\mu$ l                          | 20 $\mu$ l of 1.56 amol/ $\mu$ l  |
| 20 $\mu$ l                          | 20 $\mu$ l of 0.78 amol/ $\mu$ l  |
| 20 $\mu$ l                          | 20 $\mu$ l of 0.39 amol/ $\mu$ l  |

Prepare hybrids for blank as follows:

| <i>Volume of DNA Probe solution</i> | <i>Volume of RNase Inhibitor working solution</i> |
|-------------------------------------|---|
| 20 $\mu$ l                          | 20 $\mu$ l  |

Prepare hybrids for isolated RNA samples as follows:

| <i>Volume of DNA Probe solution</i> | <i>Volume of diluted sample</i> |
|-------------------------------------|---------------------------------|
| 20 $\mu$ l                          | 20 $\mu$ l                      |

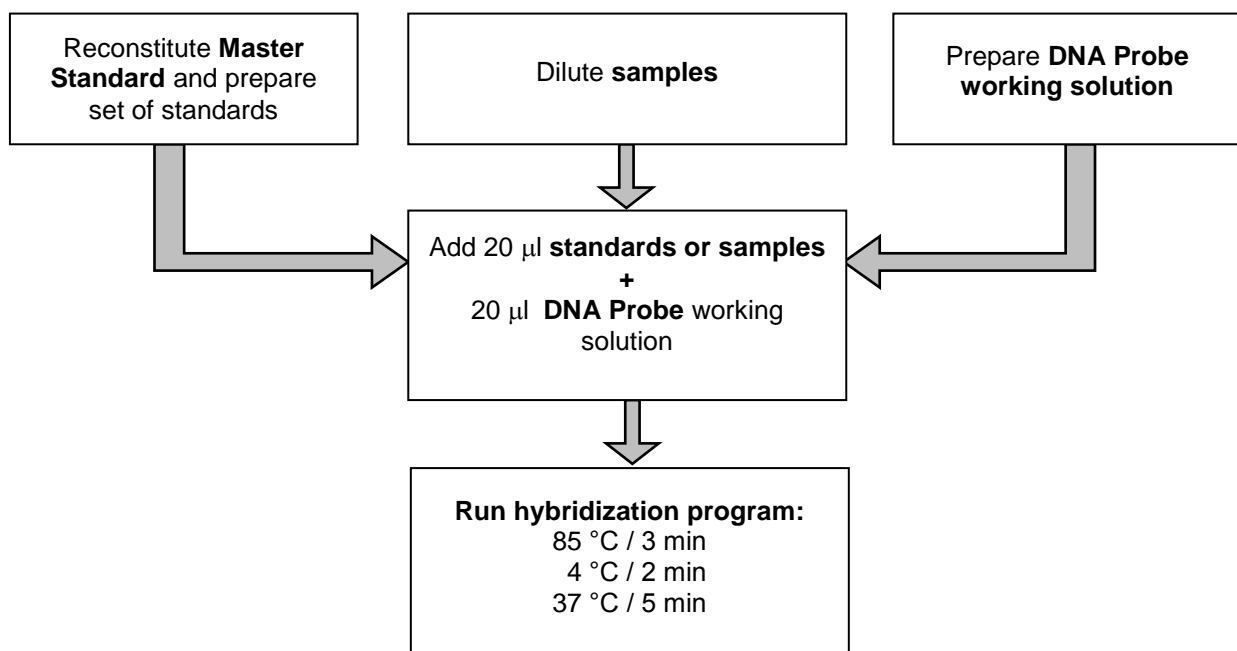
## HYBRIDIZATION PROCEDURE

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  $\mu$ l of hybrid + 70  $\mu$ l of Dilution Buffer. Mix gently (not to foam).
2. Pipet **90  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ 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 hsa-miR-126-3p 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     |
|----------|----------------------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| <b>A</b> | <b>Std.</b><br><b>12.5</b> | Sample<br>1 | Sample<br>9  | Sample<br>17 | Sample<br>25 | Sample<br>33 | Sample<br>41 | Sample<br>49 | Sample<br>57 | Sample<br>65 | Sample<br>73 | Sample<br>81 |
| <b>B</b> | <b>Std.</b><br><b>6.25</b> | Sample<br>2 | Sample<br>10 | Sample<br>18 | Sample<br>26 | Sample<br>34 | Sample<br>42 | Sample<br>50 | Sample<br>58 | Sample<br>66 | Sample<br>74 | Sample<br>82 |
| <b>C</b> | <b>Std.</b><br><b>3.13</b> | Sample<br>3 | Sample<br>11 | Sample<br>19 | Sample<br>27 | Sample<br>35 | Sample<br>43 | Sample<br>51 | Sample<br>59 | Sample<br>67 | Sample<br>75 | Sample<br>83 |
| <b>D</b> | <b>Std.</b><br><b>1.56</b> | Sample<br>4 | Sample<br>12 | Sample<br>20 | Sample<br>28 | Sample<br>36 | Sample<br>44 | Sample<br>52 | Sample<br>60 | Sample<br>68 | Sample<br>76 | Sample<br>84 |
| <b>E</b> | <b>Std.</b><br><b>0.78</b> | Sample<br>5 | Sample<br>13 | Sample<br>21 | Sample<br>29 | Sample<br>37 | Sample<br>45 | Sample<br>53 | Sample<br>61 | Sample<br>69 | Sample<br>77 | Sample<br>85 |
| <b>F</b> | <b>Std.</b><br><b>0.39</b> | Sample<br>6 | Sample<br>14 | Sample<br>22 | Sample<br>30 | Sample<br>38 | Sample<br>46 | Sample<br>54 | Sample<br>62 | Sample<br>70 | Sample<br>78 | Sample<br>86 |
| <b>G</b> | <b>Blank</b>               | Sample<br>7 | Sample<br>15 | Sample<br>23 | Sample<br>31 | Sample<br>39 | Sample<br>47 | Sample<br>55 | Sample<br>63 | Sample<br>71 | Sample<br>79 | Sample<br>87 |
| <b>H</b> | <b>Blank</b>               | Sample<br>8 | Sample<br>16 | Sample<br>24 | Sample<br>32 | Sample<br>40 | Sample<br>48 | Sample<br>56 | Sample<br>64 | Sample<br>72 | Sample<br>80 | Sample<br>88 |

*Figure 1: Example of a work sheet.*

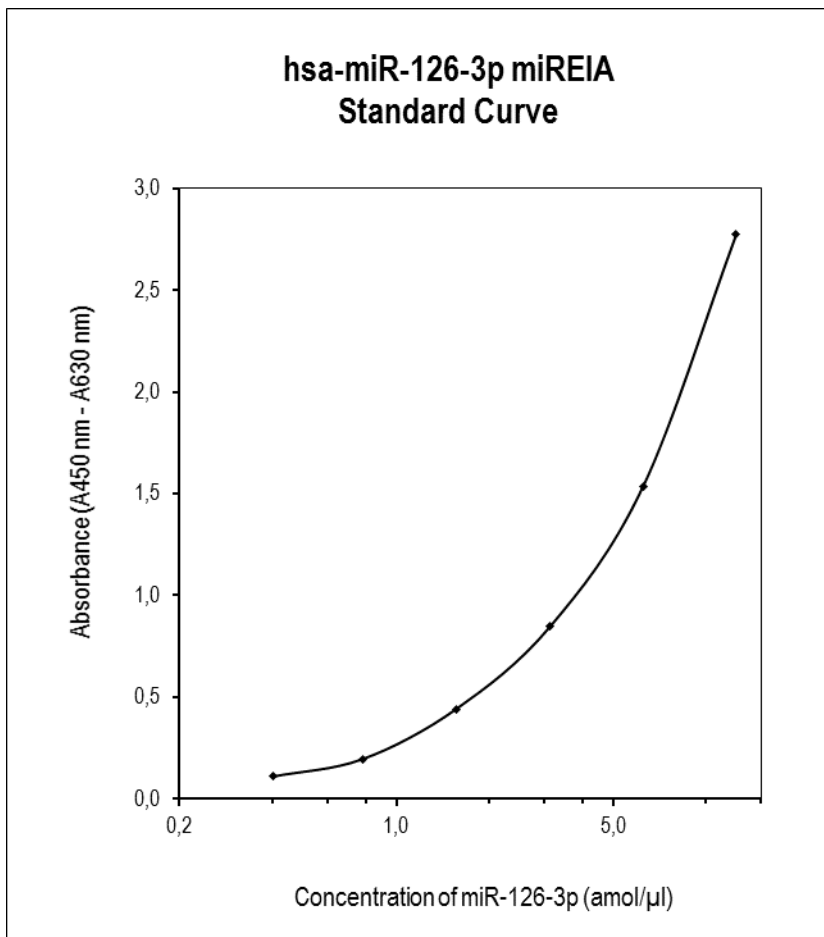


## 14. CALCULATIONS

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 hsa-miR-126-3p (amol/ $\mu$ 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/ $\mu$ l (from standard curve) x 10 (dilution factor) = 4.8 amol/ $\mu$ l.**



*Figure 2: Typical standard curve for hsa-miR-126-3p miREIA.*



## 15. PERFORMANCE CHARACTERISTICS

- **Typical analytical data of BioVendor hsa-miR-126-3p 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:  $A_{\text{blank}} + 3xSD_{\text{blank}}$  is calculated from the real hsa-miR-126-3p values in wells and is 0.13 amol/ $\mu$ l.

### 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 hsa-miR-126-3p is complementary to the sequence of hsa-miR-126-3p.

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

### Precision

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

| <i>Sample</i> | <i>Mean<br/>(amol/<math>\mu</math>l)</i> | <i>SD<br/>(amol/<math>\mu</math>l)</i> | <i>CV<br/>(%)</i> |
|---------------|--|--|-------------------|
| 1             | 3.24                                     | 0.26                                   | 8.2               |
| 2             | 0.44                                     | 0.05                                   | 10.9              |

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

| <i>Sample</i> | <i>Mean<br/>(amol/<math>\mu</math>l)</i> | <i>SD<br/>(amol/<math>\mu</math>l)</i> | <i>CV<br/>(%)</i> |
|---------------|--|--|-------------------|
| 1             | 67.59                                    | 5.55                                   | 8.2               |
| 2             | 10.80                                    | 0.80                                   | 7.4               |

## Spiking Recovery

Isolated RNA samples were diluted, spiked with different amounts of hsa-miR-126-3p and assayed.

| <i>Sample</i> | <i>Observed<br/>(amol/μl)</i> | <i>Expected<br/>(amol/μl)</i> | <i>Recovery<br/>O/E (%)</i> |
|---------------|-------------------------------|-------------------------------|-----------------------------|
| 1             | 102.81                        | -                             | -                           |
|               | 379.80                        | 341.28                        | 111.3                       |
|               | 248.93                        | 219.17                        | 113.6                       |
|               | 196.90                        | 166.81                        | 118.0                       |
| 2             | 119.90                        | -                             | -                           |
|               | 373.22                        | 358.37                        | 104.1                       |
|               | 250.03                        | 236.25                        | 105.8                       |
|               | 152.90                        | 183.90                        | 83.1                        |

## Linearity

Isolated RNA samples were serially diluted and assayed.

| <i>Sample</i> | <i>Dilution</i> | <i>Observed<br/>(amol/μl)</i> | <i>Expected<br/>(amol/μl)</i> | <i>Recovery<br/>O/E (%)</i> |
|---------------|-----------------|-------------------------------|-------------------------------|-----------------------------|
| 1             | -               | 113.30                        | -                             | -                           |
|               | 2x              | 58.22                         | 56.65                         | 102.8                       |
|               | 4x              | 29.17                         | 28.32                         | 103.0                       |
|               | 8x              | 13.06                         | 14.16                         | 92.2                        |
| 2             | -               | 129.99                        | -                             | -                           |
|               | 2x              | 73.62                         | 64.99                         | 113.3                       |
|               | 4x              | 31.97                         | 32.50                         | 98.4                        |
|               | 8x              | 16.81                         | 16.25                         | 103.5                       |

## 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 hsa-miR-126-3p levels with the assay.



## 16. DEFINITION OF THE STANDARD

The synthetic hsa-miR-126-3p is used as the standard for hsa-miR-126-3p 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, Spike-In 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






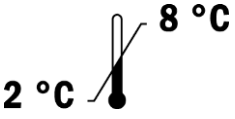

### References to hsa-miR-126-3p:

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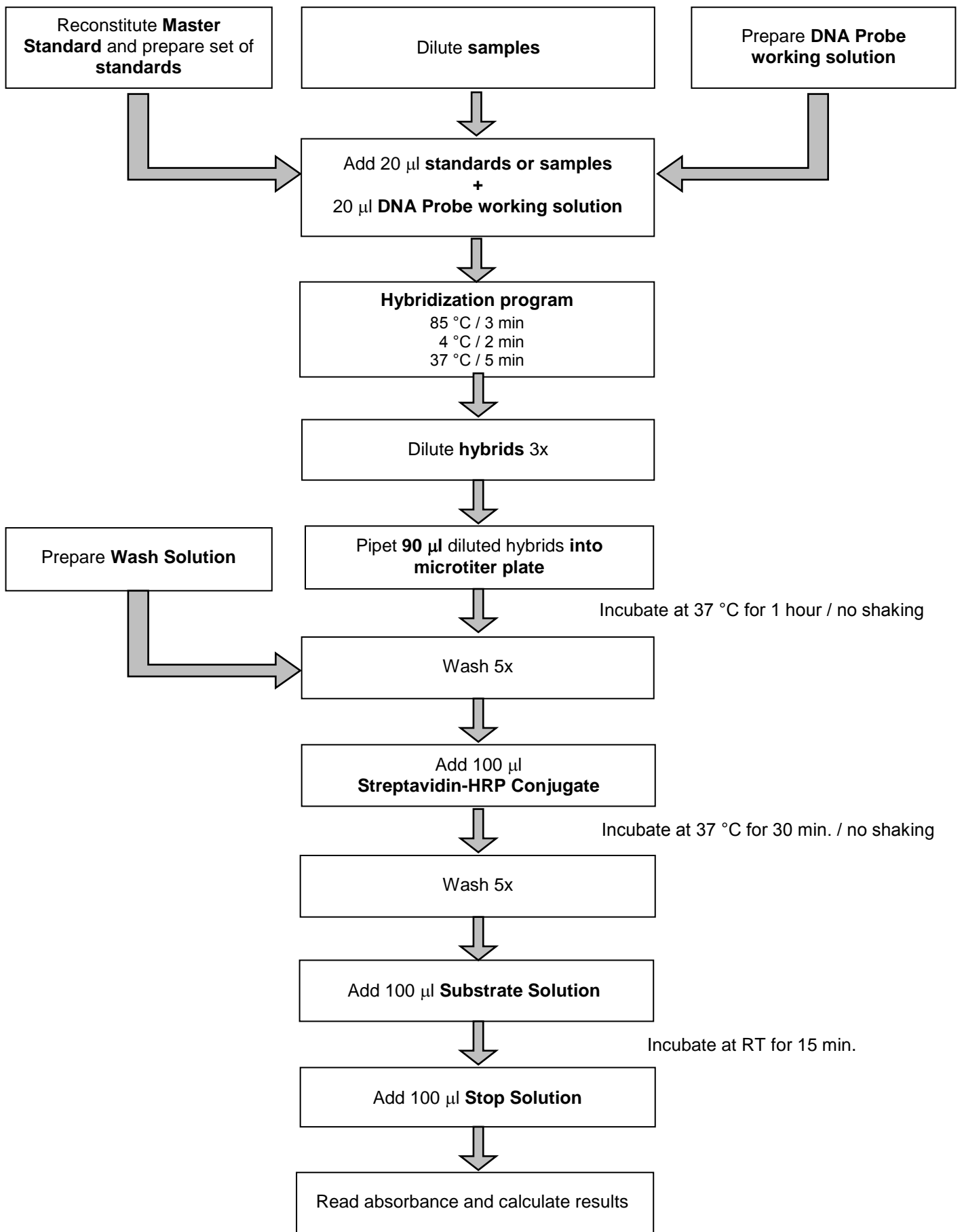
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## 19. EXPLANATION OF THE SYMBOLS

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|    | 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



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