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Glioblastoma miRNAs Diagnosis Kit

Cat. No. E20142015 (12 rnxs)

AmeriDx® Glioblastoma microRNAs Diagnosis Kit is designed for quantitative analysis of microRNAs (miRNAs) biomarkers in Glioblastoma development in human blood, Cerebrospinal Fluid (CSF), nasal mucosa or other body fluid, providing diagnosis and therapeutic guidance. The assay is sensitive, accurate and reliable.

It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.

Materials Provided

1. RNA capture tubes, 12 pcs
2. qPCR plate (384 well plate), 1 pc
3. qPCR plate film, 1 pc
4. miRNA positive control sample
5. miRNA negative control sample
6. AmeriDx® Poly (A) Polymerase, 100 uL
7. 10 mM ATP, 100 uL
8. Poly(A) Tailing Stop Buffer
9. AmeriDx® RT-qPCR mix (Cat# GS102082)
10. 10 uM primer set (10-1/ miR-320e, 10-2/miR-223, 10-3/miR-16-5p, 10-4/miR-484, 10-5/miR520a, 10-6/miR-532, 10-7/miR-630, 10-8/miR651, 10-9/miR-761)
11. Standard DNA with primers (serial diluted standard DNA template at 1, 3, 10, 33, 100, 300, 1000 pg/mL)
12. Control miRNA, 10 uL/vial, 1 vial
13. Molecular grade water, 5 x 1 mL
14. Product insert

Introduction

Glioblastoma or glioblastoma multiforme (Glioma) is an aggressive cancer that occurs mainly in the central nervous system - the brain or spinal cord. Among them, glioblastoma (GBM) is the most common intracranial primary malignant tumor. At present, the detection methods for diagnosing glioblastoma mainly include neurological function test, functional MRI and magnetic resonance spectroscopy imaging detection, and pathological evaluation (biopsy) of tissue samples. The main problem with these tests is that they are expensive and even require surgical risk. The glioblastoma miRNA rapid detection kit is based on the most advanced scientific research results of glioblastoma basic cancer, which can rapidly and accurately quantitatively detect glioblastoma-specific/abnormal concentrations in blood samples. Small molecule ribonucleic acid (miRNA). Our test kits are not only accurate, but also have unparalleled advantages in terms of cost, risk, and simplification of procedures. Therefore, we also recommend the glioblastoma miRNA rapid detection kit as a tumor marker pre-screening item in the cancer pre-screening package. Glioblastoma, also known as glioblastoma multiforme, is the most common diagnosed brain tumor in central nerve system. Glioblastoma forms from cells called astrocytes that support nerve cells. Glioblastoma can occur at any age but tends to occur more often in older adults. It can cause worsening headaches, nausea, vomiting and seizures.

Principle of the Test

AmeriDx® Glioblastoma Diagnosis Kit is a miRNA kit including all the necessary reagents for quick and accurate miRNA quantitation in biopsy. Once the miRNA is extracted, a poly(A) tail is added to the 3' end of miRNA, the miRNA with poly(A) tail is reverse transcribed with oligo(dT)-SP-primer, then SYBR PCR method is employed to quantitatively measure the copy numbers of specific miRNA.

Materials to be Provided by the End-User

- Micro centrifuge
- RNA clean-up kit (recommend AmeriDx® RNA Quick Extraction Kit, Cat# NA090720)
- Pippet (2 uL, 20 uL 200 uL, 1 mL)
- DNase-RNase free EP tubes
- Nanodrop
- qPCR instrument
- Timer
- Absorbent paper

Storage Information

- Store the kit at freezer (-20°C or below).
- Thaw the reagents at 4°C, keep the reagents on ice when use.
- Upon assay completion, return all components to appropriate storage conditions.

Specimen Collection and Handling

Blood: after collected, add RNA preservation reagents immediately, save at -80°C if not use. Serum is not recommended, due to the low yield of pure RNA.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X *g* within 30 minutes of collection. Mix with RNA preservation reagents immediately. Save at -80°C if not use.

Other body fluids or specimen, refer to specific literature for.

Reagents Prepared Before Assay

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS provided by the supplier or manufacturer.
2. Samples: For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. If dilution is required, samples should be diluted in 1X Assay Diluent before adding to the wells.

Assay Procedure

RNA Tailing

1. In a 0.2 mL PCR tube, add the following components in the order specified (set up one miRNA positive control and one, negative control sample for each batch reactions, for positive and negative control sample, use 2 uL each for 20 uL reaction volume).

Component	Volume (uL)
RNA	X
10x poly(A) Buffer	2
Water	15 - x (Add to 20 uL)
10 mM ATP	2
Poly (A) Polymerase	1
Total Volume	20

2. Incubate reaction at 37ºC for 30 minutes.
3. Clean up the RNA with RNA Clean-up Kit following the manufacturer's instruction.
4. Elute the RNA in 10 uL water, use 2 uL for concentration measurement in Nanodrop.
5. Freeze the RNA sample at -80ºC if not proceed.

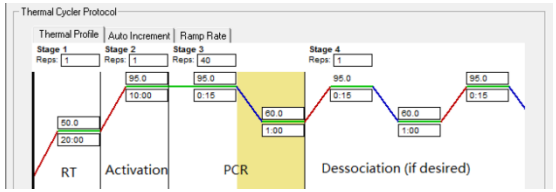
RT-qPCR

6. In a 0.2 mL PCR tube, add 5 uL of the RNA tailed with poly(A), 1 uL of oligo(dT)-SP-primer.
7. Put the tube on thermal cycler, heat at 65ºC for 5 minutes, transfer to ice immediately after 65ºC incubation.

8. Add the RT-qPCR buffer and other components to each of the sample tube.

Component	1x (uL)
2x RT-qPCR Master Mix	10
PCR Primer Mix	2
Water	2
Total Volume	14

9. Transfer to 384 well plate, write down the sample position (layout).
10. Similarly make standard curve sample, use Standard DNA and Standard primer set.
11. Load 384 well plate on 7900HT PCR instrument, se the program and run the assay.



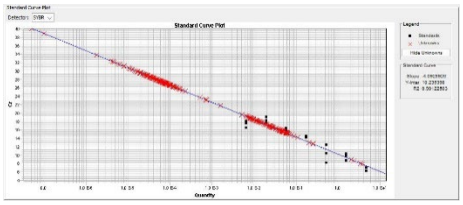
12. Analyze the qPCR data by pasting the data to designated position on the data analysis Excel template.

Data Analysis

1. Export the data from qPCR instrument (eg. ABI 7900 HT) as .txt document.
2. Import to Microsoft Excel sheet, analyze the data with Excel Data Analysis Template for miRNA quantitation.

Standard Curve

qPCR Standard Curve: This standard curve is generated from an example; individual experiment may vary.



Assay Procedure Summary

Performance Characteristics

Sensitivity: The minimum detectable concentration of human miRNA is xx copies/mL sample (based on Mean of 20 negative control wells plus 2 Standard Deviation).

Specificity: No cross reactivity was checked.

Troubleshooting

High Background:

1. Background wells were contaminated.
2. Assay buffer is contaminated with miRNA.

No signal:

3. RNA poly(A) tailing failed
4. cDNA synthesis failed.

Low or poor signal for the standard curve:

5. Standard was incomplete or was stored improperly.
6. Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

7. Standard was incompletely reconstituted or was stored improperly.
8. Reagents were added to wells with incorrect concentrations.
9. Plate was incubated with improper temperature, timing, or agitation.

Signal too high, standard curves saturated:

10. Standard was reconstituted with less volume than required.
11. One or more reagent incubation steps were too long.
12. Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

13. Samples contain no or below detectable levels of antibody.
14. Samples contain antibody concentrations greater than highest standard point.

High variations in samples and/or standards:

15. Pipetting errors may have occurred.
16. Plate washing was inadequate or no uniform.
17. Samples were not homogenous.
18. Samples or standard wells were contaminated.

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