**Bioingentech Genomic RNA Purification** **Rev. Date: February 2017**

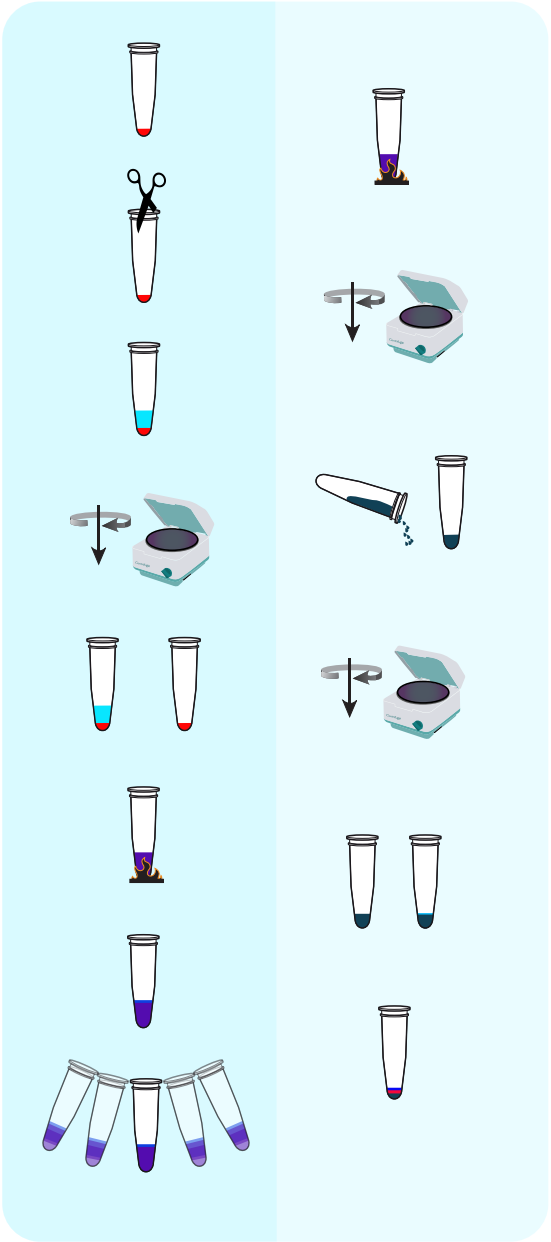
RNA pathogens detection

| PCR cycling parameters used | TIME |
|---|---|
| 1 cycle Initial Denaturation 94°C | 3 min |
| 30 cycles | |
| Denaturation 94°C | 30 sec |
| Annealing 57°C | 30 sec |
| Extension 72°C | 30 sec |
| 1 cycle Final extension 72°C | 5 min |

## RNA Extraction from Shrimp

### Samples: Hepatopancreas, Gills and Pleopods.

1. Cut the samples and fill the tube until approximately 200 uL mark in a 2000 uL tube.
2. Macerate the sample with scissor.
3. Add 1 mL of CLS to wash the sample.
4. Centrifuge to 14.000 RPM for 3 minutes then discard supernatant
5. Add 250 µL RNA lysis buffer, homogenize and incubate for 10 min at room temperature.
6. Add 50 µL of chloroform.
7. Shake tube vigorously by hand for 15 seconds and vortex the sample briefly.
8. Incubate for 10 min at -20ºC.
9. Centrifuge the sample at 12000 x g for 15 minutes.
10. Remove 45 µL of upper aqueous phase into a new tube and add 45 µL of Isopropanol, homogenize and incubate for 10 minutes -20ºC.
11. Centrifuge at 12000 x g for 10 minutes.
12. Remove the supernatant (70µL) and wash the pellet with 45 µL of RNA Wash solution
13. Centrifuge the tube at 12000 x g for 5 min and discard the wash.
14. Resuspend the RNA pellet in RNA rehydration solution (5-10µL)
15. Add 2 µL of RNA of sample in (4,5 µL Vet PCR RT PCR Premixture+ 4 µL DNase/RNase free water) *



*See Components PCR Kit: Vet PCR operation Manual Bioingentech, TABLE 1. www.kitpcr.com

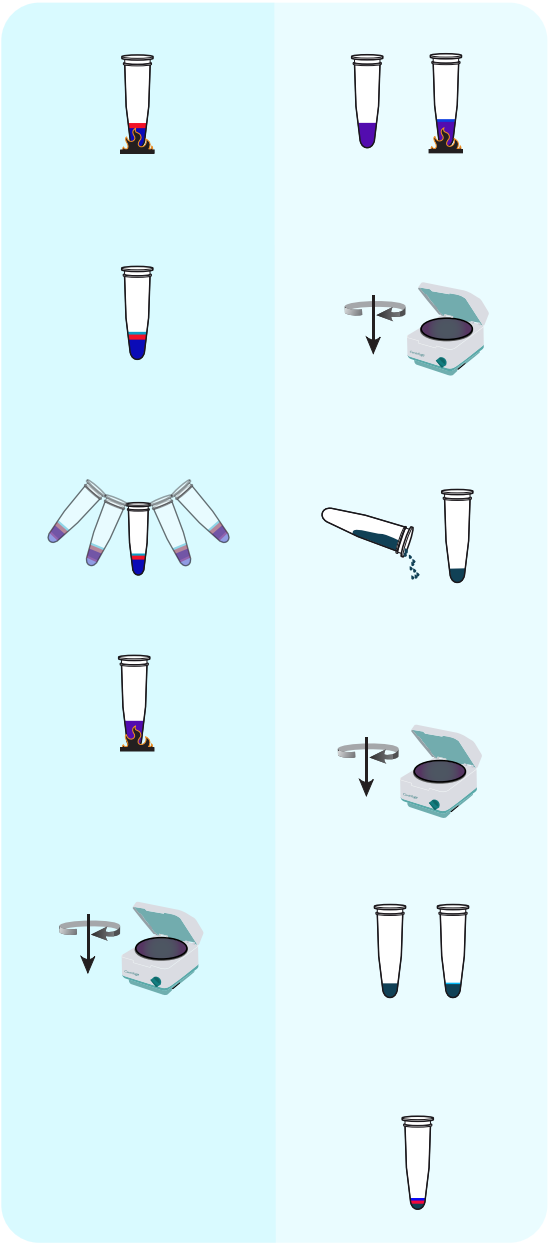
## RNA Lysis Buffer Bioingentech REAGENT

Reagent is ready to use

## A. Preparing whole blood with EDTAK3 samples: RNA isolation procedure

### A.1 RNA extracted from 62 µL of blood, using 250 RNA lysis buffer Bioingentech

1. Add 250 µL RNA lysis buffer per 62 µL of blood, homogenize and incubate for 10 min at room temperature.
2. Add 50 µL of chloroform.
3. Shake tube vigorously by hand for 15 seconds and vortex the sample briefly.
4. Incubate for 10 min at -20ºC.
5. Centrifuge the sample at 12000 x g for 15 minutes.
6. Remove 45 µL of upper aqueous phase into a new tube and add 45 µL of Isopropanol, homogenize and incubate for 10 minutes -20ºC.
7. Centrifuge at 12000 x g for 10 minutes.
8. Remove the supernatant (70 µL) and wash the pellet with 45 µL of RNA Wash solution.
9. Centrifuge the tube at 12000 x g for 5 min and discard the wash.
10. Resuspend the RNA pellet in RNA rehydration solution (3µL).
11. Add 2 µL of RNA of sample in (4,5 µL Vet PCR RT PCR Premixture+ 4 µL DNase/ RNase free water) *



*See Components PCR Kit: Vet PCR operation Manual Bioingentech, TABLE 1. www.kitpcr.com

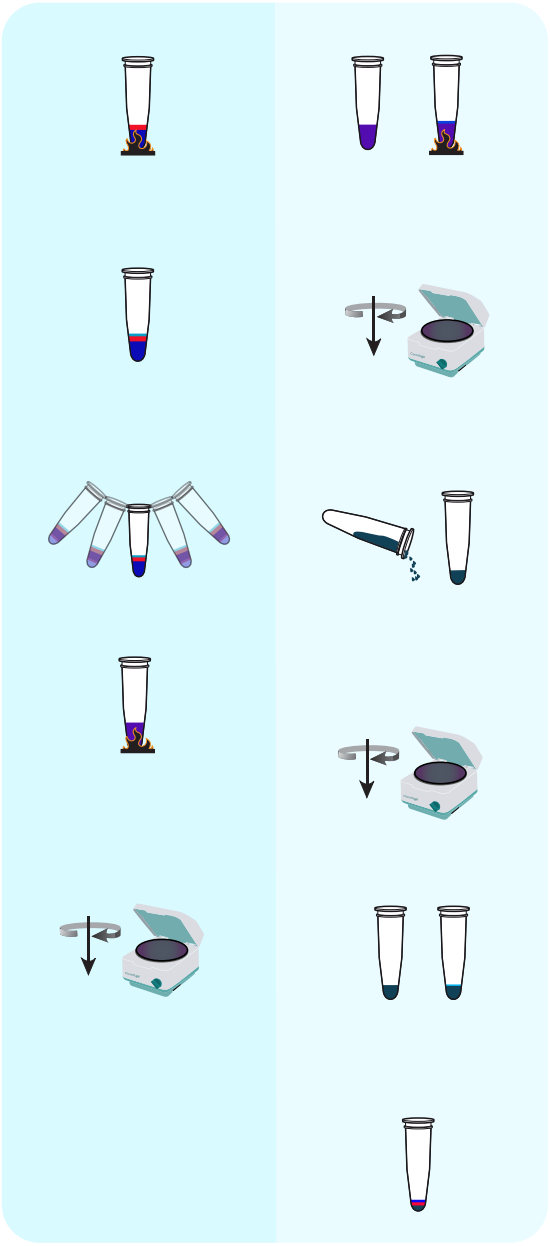
## RNA Lysis Buffer Bioingentech REAGENT

Reagent is ready to use

## A. Preparing whole blood with EDTAK3 samples: RNA isolation procedure

A.2 RNA extracted from 125 µL of blood, using 500 µL RNA lysis buffer Bioingentech

1. Add 500 µL RNA lysis buffer per 125 µL of blood, homogenize and incubate for 10 min at room temperature.
2. Add 100 µL of chloroform.
3. Shake tube vigorously by hand for 15 seconds and vortex the sample briefly.
4. Incubate for 10 min at -20ºC.
5. Centrifuge the sample at 12000 x g for 15 minutes.
6. Remove 90 µL of upper aqueous phase into a new tube and add 90 µL of Isopropanol, homogenize and incubate for 10 minutes -20ºC.
7. Centrifuge at 12000 x g for 10 minutes.
8. Remove the supernatant (160 µL) and wash the pellet with 90 µL of RNA Wash solution.
9. Centrifuge the tube at 12000 x g for 5 min and discard the wash.
10. Resuspend the RNA pellet in RNA rehydration solution (5µL).
11. Add 2 µL of RNA of sample in (4,5 µL Vet PCR RT PCR Premixture+ 4 µL DNase/ RNase free water) *



*See Components PCR Kit: Vet PCR operation Manual Bioingentech, TABLE 1. www.kitpcr.com

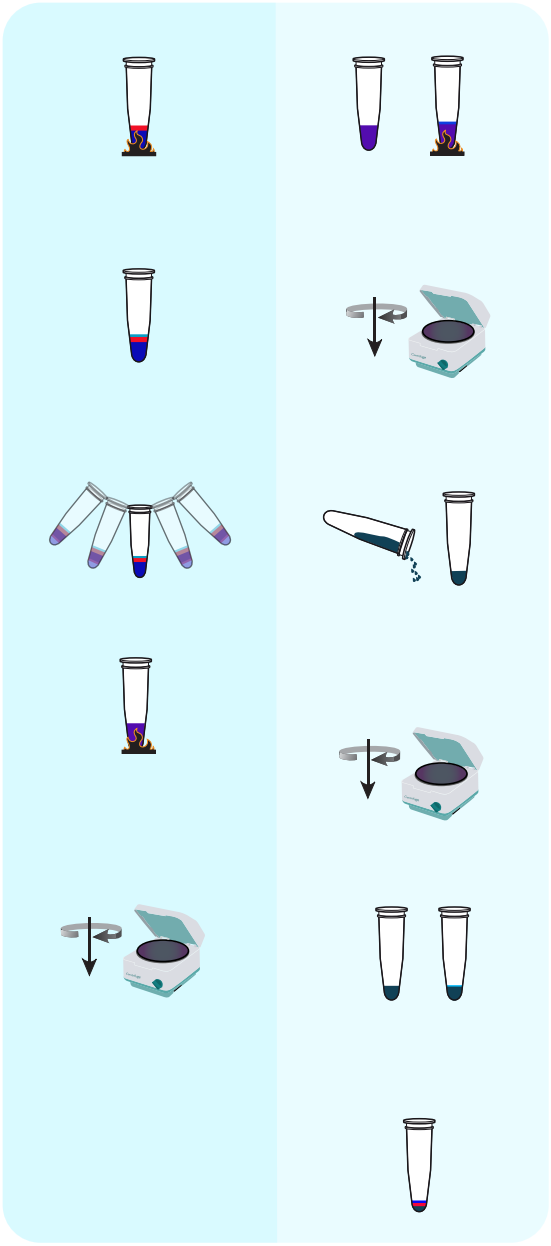
## RNA Lysis Buffer Bioingentech REAGENT

Reagent is ready to use

## A. Preparing whole blood with EDTAK3 samples: RNA isolation procedure

### A.3 RNA extracted from 250 µL of blood, using 1mL RNA lysis buffer Bioingentech

1. Add 1mL RNA lysis buffer Bioingentech per 250 µL of blood, homogenize and incubate for 10 min at room temperature.
2. Add 200 µL of chloroform.
3. Shake tube vigorously by hand for 15 seconds and vortex the sample briefly.
4. Incubate for 10 min at -20ºC.
5. Centrifuge the sample at 12000 x g for 15 minutes.
6. Remove 180 µL of upper aqueous phase into a new tube and add 180 µL of Isopropanol, homogenize and incubate for 10 minutes -20ºC.
7. Centrifuge at 12000 x g for 10 minutes.
8. Remove the supernatant (340 µL) and wash the pellet with 180 µL of RNA Wash solution.
9. Centrifuge the tube at 12000 x g for 5 min and discard the wash.
10. Resuspend the RNA pellet in RNA rehydration solution (10µL).
11. Add 2 µL of RNA of sample in (4,5 µL Vet PCR RT PCR Premixture+ 4 µL DNase /RNase free water) *



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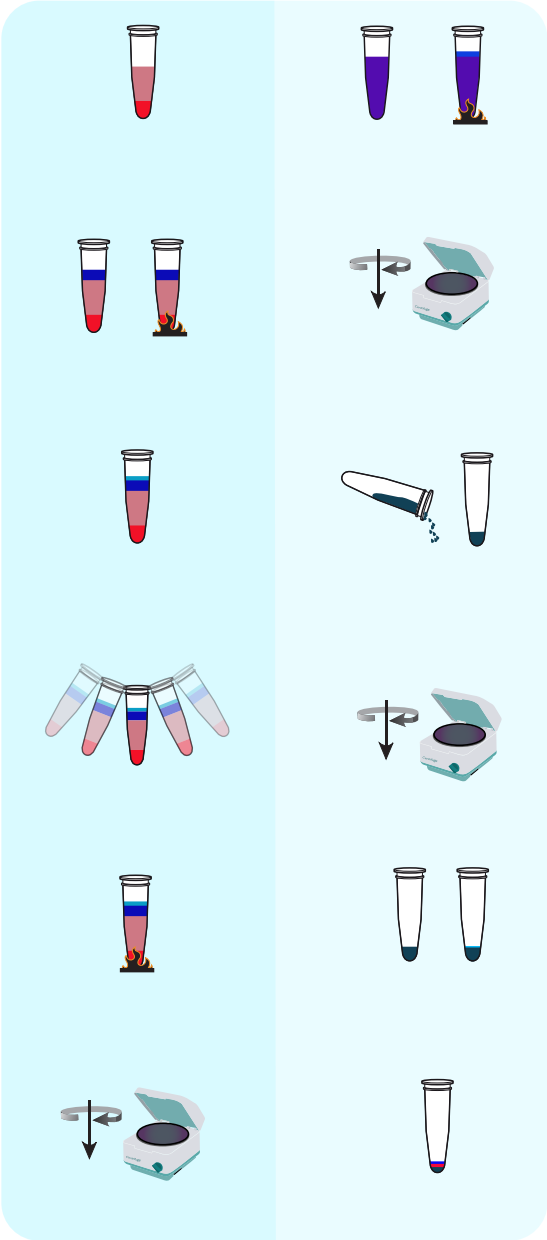
## RNA Lysis Buffer Bioingentech REAGENT

Reagent is ready to use

## B.-Preparing white blood cells: RNA isolation procedure

RNA extracted from white blood cells, using 300µL RNA lysis buffer Bioingentech

1. Add 1 mL cell Lysis Bioingentech per 300 µl of blood, homogenize and Centrifuge the sample at 12.000 x g for 5 minutes. Remove the supernatant and then repeat the first step.
2. Add 300 µl RNA lysis buffer Bioingentech per pellet, homogenize and incubate for 10 min at room temperatu-re. for 10 min at room temperature.
3. Add 100 µL of chloroform.
4. Shake tube vigorously by hand for 15 seconds and vortex the sample briefly.
5. Incubate for 10 min at -20ºC.
6. Centrifuge the sample at 12000 x g for 15 minutes.
7. Remove 80 µL of upper aqueous phase into a new tube and add 120 µL of Isopropanol, homogenize and incubate for 10 minutes -20ºC.
8. Centrifuge at 12000 x g for 10 minutes.
9. Remove the supernatant (180 µL) and wash the pellet with 150 µL of RNA Wash solution.
10. Centrifuge the tube at 12000 x g for 5 min and discard the wash.
11. Resuspend the RNA pellet in RNA rehydration solution (10µL).
12. Add 2 µL of RNA of sample in (4,5 µL Vet PCR RT PCR Premixture+ 4 µL DNase/RNase free water) *



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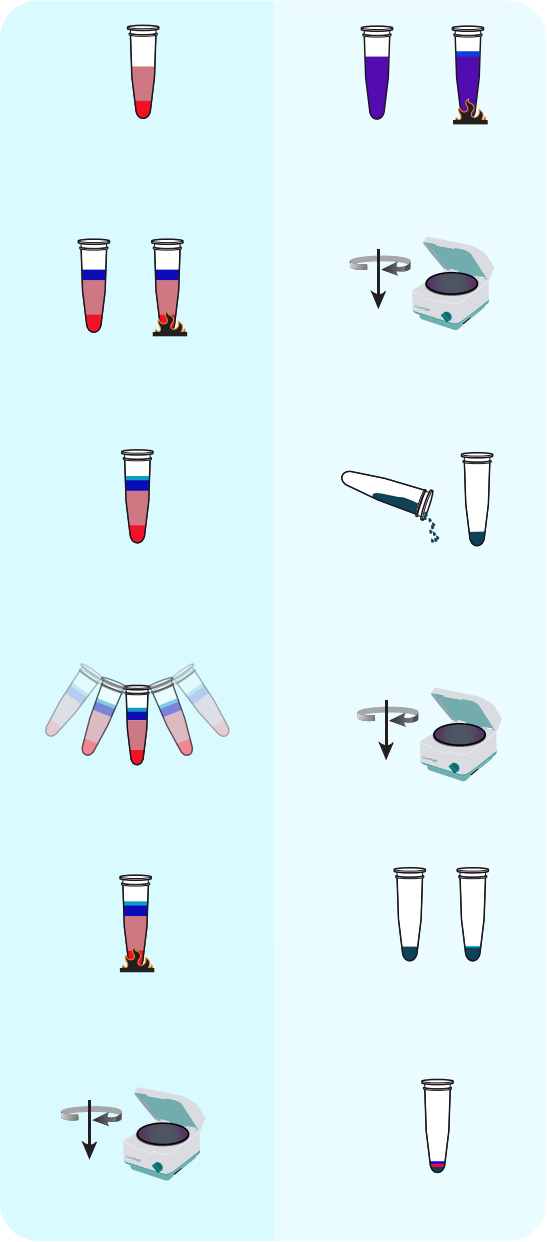
## RNA Lysis Buffer Bioingentech REAGENT

Reagent is ready to use

## C.-Preparing serum samples: RNA isolation procedure

RNA extracted from SERUM, using 300µL RNA lysis buffer Bioingentech

1. Centrifuge 1,5 mL serum sample at 12.000 x g for 5 minutes. Remove the supernatant.
2. Add 300 µl RNA lysis buffer Bioingentech per pellet, homogenize and incubate for 10 min at room temperature.
3. Add 100 µL of chloroform.
4. Shake tube vigorously by hand for 15 seconds and vortex the sample briefly.
5. Incubate for 10 min at -20ºC.
6. Centrifuge the sample at 12000 x g for 15 minutes.
7. Remove 80 µL of upper aqueous phase into a new tube and add 120 µL of Isopropanol, homogenize and incubate for 10 minutes -20ºC.
8. Centrifuge at 12000 x g for 10 minutes.
9. Remove the supernatant (180 µL) and wash the pellet with 150 µL of RNA Wash solution.
10. Centrifuge the tube at 12000 x g for 5 min and discard the wash.
11. Resuspend the RNA pellet in RNA rehydration solution (6µL).
12. Add 2 µL of RNA of sample in (4,5 µL Vet PCR RT PCR Premixture+ 4 µL DNase/RNase free water) *



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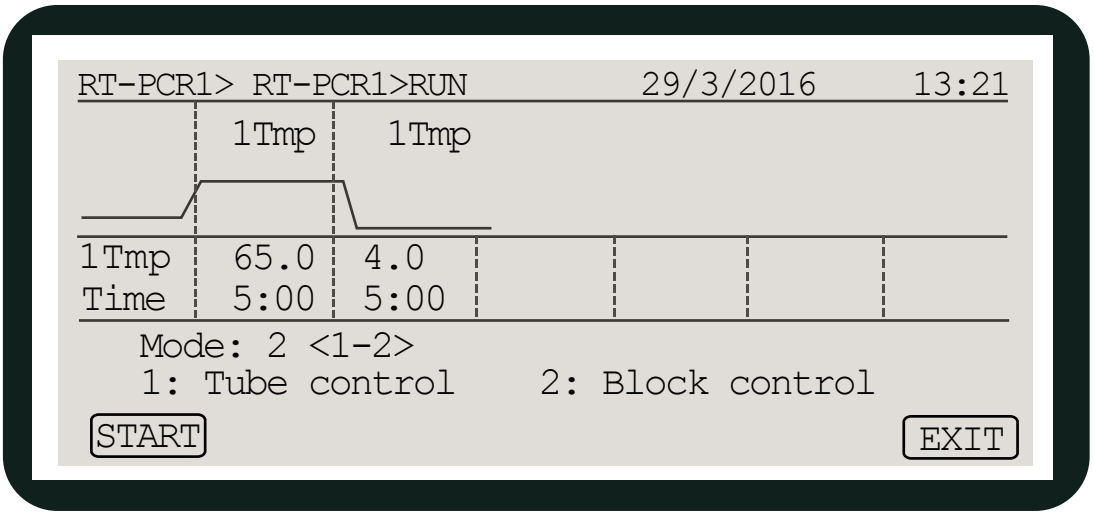
## Conditions used and suggested of RT- PCR to obtain complementary DNA (cDNA) RT-PCR-1




## Add 1,0 µL of Brig RT-PCR Solution and 1,0 µL of Biotech transcriptase solution RT-PCR-2