# Overview of
# Standard protocol

S

Sample types: blood, plasma, serum, milk, oral fluids, ear notch in pigs, tissue homogenized in PBS

## 1. Sample Lysis

- ✔ 200 µL sample
- ✔ 180 µL SLS
- ✔ 20 µL Proteinase K
- ✔ 4 µL carrier RNA (recommended)
- ✔ x µL Extraction control (recommended)

**1.1.** Mix by pipetting

**1.2.** Add entire lysate to Step 2 Binding Mix

## 2. Binding of nucleic acids to Beads

- ✔ 600 µL Binding Buffer
- ✔ 20 µL Beads

**2.1.** Mix by shaking for 5-10 min at RT (optional: mix by pipetting)

**2.2.** Remove supernatant after 2 min magnetic separation

## 3. Washing with Wash Buffer 1

- ✔ 600 µL Wash Buffer 1

**3.1.** Resuspend: Shake for 1-3 min at RT

**3.2.** Remove supernatant after 2 min magnetic separation

## 4. Washing with Wash Buffer 2

- ✔ 600 µL Wash Buffer 2

**4.1.** Resuspend: Shake for 1-3 min at RT

**4.2.** Remove supernatant after 2 min magnetic separation

## 5. Washing with 80% Ethanol

- ✔ 600 µL 80% Ethanol

**5.1.** Resuspend: Shake for 1-3 min at RT

**5.2.** Remove supernatant after 2 min magnetic separation

## 6. Air-drying of Beads

**6.1.** Air dry for 10 min at RT

## 7. Elution of RNA/DNA

- ✔ 50-100 µL Elution Buffer

**7.1.** Resuspend: Shake for 5 min at RT (optional: mix by pipetting)

**7.2.** Transfer RNA/DNA into elution plate or tube after 2 min magnetic separation

vmrd

for Viral Nucleic Acids Bacterial Nucleic Acids

# Overview of
# Enhanced protocol

E

Sample types: blood, plasma, serum, milk, oral fluids, ear notch in pigs, tissue homogenized in PBS

## 1. Sample Lysis

- ✔ 200 µL sample
- ✔ 350 µL ELS
- ✔ 150 µL PBS
- ✔ 7 µL carrier RNA (recommended)
- ✔ x µL Extraction control (recommended)
- ✔ 1 vial Lysis Beads

**1.1.** Bead beating for 15 min.

**1.2.** centrifugation at 16,000 x g for 5 min.

**1.3.** Add 405 µL clarified lysate to Step 2 Binding Mix

## 2. Binding of nucleic acids to Beads

- ✔ 600 µL Binding Buffer
- ✔ 20 µL Proteinase K
- ✔ 20 µL Beads

**2.1.** Mix by shaking for 5-10 min at RT (optional: mix by pipetting)

**2.2.** Remove supernatant after 2 min magnetic separation

## 3. Washing with Wash Buffer 1

- ✔ 600 µL Wash Buffer 1

**3.1.** Resuspend: Shake for 1-3 min at RT

**3.2.** Remove supernatant after 2 min magnetic separation

## 4. Washing with Wash Buffer 2

- ✔ 600 µL Wash Buffer 2

**4.1.** Resuspend: Shake for 1-3 min at RT

**4.2.** Remove supernatant after 2 min magnetic separation

## 5. Washing with 80% Ethanol

- ✔ 600 µL 80% Ethanol

**5.1.** Resuspend: Shake for 1-3 min at RT

**5.2.** Remove supernatant after 2 min magnetic separation

## 6. Air-drying of Beads

**6.1.** Air dry for 10 min at RT

## 7. Elution of RNA/DNA

- ✔ 50-100 µL Elution Buffer

**7.1.** Resuspend: Shake for 5 min at RT (optional: mix by pipetting)

**7.2.** Transfer RNA/DNA into elution plate or tube after 2 min magnetic separation

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for Nucleic Acids from difficult to lyse bacteria

# Overview of
# Modified protocol

M

Sample types: ear notch in SLS, tissue homogenized in SLS, ear notch in ELS, tissue homogenized in ELS

## 1. Sample Lysis

- Tissue section
- 200 µL SLS or ELS
- 4 µL carrier RNA

**1.1.** Incubation for minimum 15 min

**1.2.** Add entire lysate to Step 2 Binding Mix

## 2. Binding of nucleic acids to Beads

- 600 µL Binding Buffer
- 20 µL Proteinase K
- 20 µL Beads
- x µL extraction control (recommended)

**2.1.** Mix by shaking for 5-10 min at RT (optional: mix by pipetting)

**2.2.** Remove supernatant after 2 min magnetic separation

## 3. Washing with Wash Buffer 1

- 600 µL Wash Buffer 1

**3.1.** Resuspend: Shake for 1-3 min at RT

**3.2.** Remove supernatant after 2 min magnetic separation

## 4. Washing with Wash Buffer 2

- 600 µL Wash Buffer 2

**4.1.** Resuspend: Shake for 1-3 min at RT

**4.2.** Remove supernatant after 2 min magnetic separation

## 5. Washing with 80% Ethanol

- 600 µL 80% Ethanol

**5.1.** Resuspend: Shake for 1-3 min at RT

**5.2.** Remove supernatant after 2 min magnetic separation

## 6. Air-drying of Beads

**6.1.** Air dry for 10 min at RT

## 7. Elution of RNA/DNA

- 50-100 µL Elution Buffer

**7.1.** Resuspend: Shake for 5 min at RT (optional: mix by pipetting)

**7.2.** Transfer RNA/DNA into elution plate or tube after 2 min magnetic separation

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for Viral Nucleic Acids

# Overview of
# High Inhibitors protocol

HB

Sample types: feces

## 1. Sample Lysis

- ✔ 140 µL of clarified feces
- ✔ 350 µL Enhanced Lysis Solution
- ✔ 210 µL 1X PBS
- ✔ 7 µL Carrier RNA (recommended)
- ✔ x µL extraction control (recomm.)
- ✔ 1 vial Lysis Beads

**1.1.** Bead-beating for 15 min

**1.2.** Centrifugation at 16,000 x g for 5 min

**1.3.** Add 405 µL clarified lysate to Step 2 Binding Mix

## 2. Binding of nucleic acids to Beads

- ✔ 600 µL Binding Buffer
- ✔ 20 µL Beads
- ✔ 20 µL Proteinase K

**2.1.** Mix by shaking for 5-10 min at RT (optional: mix by pipetting)
**2.2.** Remove supernatant after 2 min magnetic separation

## 3. Washing with Wash Buffer 1

- ✔ 600 µLWash Buffer 1

**3.1.** Resuspend: Shake for 1-3 min at RT
**3.2.** Remove supernatant after 2 min magnetic separation

## 4. Washing with Wash Buffer 2

- ✔ 600 µlLWash Buffer 2

**4.1.** Resuspend: Shake for 1-3 min at RT
**4.2.** Remove supernatant after 2 min magnetic separation

## 5. Washing with 80% Ethanol

- ✔ 600 µL 80% Ethanol

**5.1.** Resuspend: Shake for 1-3 min at RT
**5.2.** Remove supernatant after 2 min magnetic separation

## 6. Air-drying of Beads

**6.1.** Air dry for 10 min at RT

## 7. Elution of RNA/DNA

- ✔ 50-100 µL Elution Buffer

**7.1.** Resuspend: Shake for 5 min at RT (optional: mix by pipetting)
**7.2.** Transfer RNA/DNA into elution plate or tube after 2 min magnetic separation

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for Nucleic Acids from Difficult to Lyse Bacteria

# Overview of
# High Inhibitors Viral protocol

HV

Sample types: feces

## 1. Sample Lysis

- 140 µL of clarified feces
- 350 µL Enhanced Lysis Solution
- 7 µL Carrier RNA (recommended)
- x µL extraction control (recomm.)
- 1 vial Lysis Beads

**1.1.** Mix vigorously by pipetting or vortexing

**1.2.** Centrifugation at 16,000 x g for 5 min

**1.3.** Add 405 µL clarified lysate to Step 2 Binding Mix

## 2. Binding of nucleic acids to Beads

- 600 µL Binding Buffer
- 20 µL Beads
- 20 µL Proteinase K

**2.1.** Mix by shaking for 5-10 min at RT (optional: mix by pipetting)
**2.2.** Remove supernatant after 2 min magnetic separation

## 3. Washing with Wash Buffer 1

- 600 µLWash Buffer 1

**3.1.** Resuspend: Shake for 1-3 min at RT
**3.2.** Remove supernatant after 2 min magnetic separation

## 4. Washing with Wash Buffer 2

- 600 µlLWash Buffer 2

**4.1.** Resuspend: Shake for 1-3 min at RT
**4.2.** Remove supernatant after 2 min magnetic separation

## 5. Washing with 80% Ethanol

- 600 µL 80% Ethanol

**5.1.** Resuspend: Shake for 1-3 min at RT
**5.2.** Remove supernatant after 2 min magnetic separation

## 6. Air-drying of Beads

**6.1.** Air dry for 10 min at RT

## 7. Elution of RNA/DNA

- 50-100 µL Elution Buffer

**7.1.** Resuspend: Shake for 5 min at RT (optional: mix by pipetting)
**7.2.** Transfer RNA/DNA into elution plate or tube after 2 min magnetic separation

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for Viral Nucleic Acids

# Overview of
# High Extenders protocol

HE

Sample types: semen

## 1. Sample Lysis

- ✔ 200 µL semen sample
- ✔ 200 µL ELS
- ✔ 20 µL Proteinase K
- ✔ 4 µL carrier RNA (recommended)

**1.1.** Incubate at RT for 15 min (optional: Incubate at 60˚C for 15 min)
**1.2.** Centrifugation at 15,000 x g for 1 min
**1.3.** Add entire lysate to Step 2 Binding Mix

## 2. Binding of nucleic acids to Beads

- ✔ 600 µL Binding Buffer
- ✔ 20 µL Beads
- ✔ x µL extraction control (recommended)

**2.1.** Mix by shaking for 5-10 min at RT (optional: mix by pipetting)
**2.2.** Remove supernatant after 2 min magnetic separation

## 3. Washing with Wash Buffer 1

- ✔ 600 µL Wash Buffer 1

**3.1.** Resuspend: Shake for 1 min at RT
**3.2.** Remove supernatant after 2 min magnetic separation

## 4. Washing with Wash Buffer 2

- ✔ 600 µL Wash Buffer 2

**4.1.** Resuspend: Shake for 1 min at RT
**4.2.** Remove supernatant after 2 min magnetic separation

## 5. Washing with 80% Ethanol

- ✔ 600 µL 80% Ethanol

**5.1.** Resuspend: Shake for 1 min at RT
**5.2.** Remove supernatant after 2 min magnetic separation

## 6. Air-drying of Beads

**6.1.** Air dry for 10 min at RT

## 7. Elution of RNA/DNA

- ✔ 50-100 µL Elution Buffer

**7.1.** Resuspend: Shake for 5 min at RT (optional: mix by pipetting)
**7.2.** Transfer RNA/DNA into elution plate or tube after 2 min magnetic separation

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for Viral and Bacterial nucleic acids