Panther Fusion® Extraction Reagents-X

For in vitro diagnostic use.

Intended Use ................................................................. 2
Principles of the Procedure ........................................... 2
Materials Provided ....................................................... 2
Materials Required and Available Separately ................... 2
Warnings and Precautions ............................................... 3
Storage and Handling Requirements ............................... 4
Sample Preparation ......................................................... 5
  Definitions ........................................................................ 5
  Notes ............................................................................. 5
  Preparation of Working Diluents ..................................... 6
  Nasopharyngeal (NP) Swab in VTM and Nasal Swab in Liquid Amies Specimen Processing ........................................... 6
  Lower Respiratory Tract (LRT) Specimen Processing ........ 6
  EDTA Plasma and Serum Specimen Processing .................. 6
  EDTA Whole Blood Specimen Processing .......................... 7
  Stool Specimen Processing ............................................. 7
  Urine Specimen Processing ............................................. 7
  Cerebrospinal Fluid (CSF) Specimen Processing ............... 7
  ThinPrep Specimen Processing ......................................... 7
  Vaginal, Endocervical, Rectal, Throat, and Lesion Swab Specimen Processing ....................................................... 8
  Lim or Carrot Broth Culture Specimen Processing .............. 8
Panther Fusion System Test Procedure ............................... 8
  Work Area Preparation ................................................... 8
  Reagent Preparation ...................................................... 8
  Specimen Handling ....................................................... 8
  System Preparation ....................................................... 9
Limitations ........................................................................... 9
Intended Use

The Panther Fusion® Extraction Reagents-X are intended for extraction of DNA from nasopharyngeal, lower respiratory tract (bronchial wash and bronchoaveolar lavage), EDTA plasma, serum, EDTA whole blood, stool, urine, cerebral spinal fluid (CSF), ThinPrep samples, vaginal swabs, endocervical swabs, rectal swabs, throat swabs, skin lesions, Lim broth cultures, and Carrot broth cultures using upstream sample extraction capabilities of the Panther Fusion System®.

Principles of the Procedure

Prior to processing and testing on the Panther Fusion system, prepare specimens as described in this document. The internal control target present in the Internal Control-X (IC-X) reagent is added to each test specimen via the working Panther Fusion Capture Reagent-X (wFCR-X). The IC-X in the reagent may be used to monitor specimen processing, amplification and detection. Capture oligonucleotides hybridize to nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube. The elution step elutes purified nucleic acid. During the nucleic acid capture and elution step, DNA is isolated from specimens.

Refer to the Panther Fusion assay package inserts for specific information on sample preparation for approved assays. Refer to the Panther Fusion Operator's Manual for information on the operation of the Panther Fusion System.

Materials Provided

Panther Fusion Extraction Reagents-X (Cat No. PRD-04477)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Volume</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panther Fusion Capture Reagent-X</td>
<td>4 x 240</td>
<td>173 mL/bottle</td>
<td>A buffered salt solution containing solid phase (magnetic particles) and non-infectious nucleic acids</td>
</tr>
<tr>
<td>Panther Fusion Enhancer Reagent-X</td>
<td>4 x 240</td>
<td>70 mL/bottle</td>
<td>An alkaline solution of lithium hydroxide</td>
</tr>
</tbody>
</table>

Materials Required and Available Separately

Note: Materials available from Hologic have catalog numbers listed, unless otherwise specified.

<table>
<thead>
<tr>
<th>Component</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panther System</td>
<td>303095</td>
</tr>
<tr>
<td>Panther Fusion Module</td>
<td>PRD-04173</td>
</tr>
<tr>
<td>Panther Fusion System</td>
<td>PRD-04172</td>
</tr>
<tr>
<td>Panther Fusion Internal Control-X 960 Tests</td>
<td>PRD-04476</td>
</tr>
<tr>
<td>Panther Fusion Specimen Lysis Tubes (SLT), 100 per bag</td>
<td>PRD-04339</td>
</tr>
<tr>
<td>Aptima Penetrable Caps</td>
<td>105668</td>
</tr>
<tr>
<td>Transport Tube, Polypropylene, 50 per bag</td>
<td>401457</td>
</tr>
<tr>
<td>Specimen Aliquot Tubes (SAT), 100 pack</td>
<td>503762</td>
</tr>
</tbody>
</table>
Warnings and Precautions

Aptima Multitest Swab Specimen Collection Kit PRD-03546
Aptima Specimen Transfer Kit 301154C
Specimen Transport Medium (STM) PRD-04423
Urine Transport Medium (UTM) PRD-04943
Viral Transport Medium (VTM)
  VTM verified for use:
    Remel MicroTest M4, M4RT, M5 or M6 formulation
    Copan Universal Transport Medium
    BD Universal Viral Transport Medium
Blood Transport Medium (BTM) PRD-04944
Open Access Diluent Additive PRD-04945
Proteinase K
Phosphate Buffered Saline (PBS)

Warnings and Precautions

A. Use routine laboratory precautions. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling reagents.

B. Avoid microbial and ribonuclease contamination of reagents.

C. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

D. Store reagents at the recommended storage condition. See Storage and Handling Requirements.

E. The Panther Fusion Enhancer Reagent-X (FER-X) is corrosive, harmful if swallowed and causes severe skin burns and eye damage.

F. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.

G. Do not use the reagents after the expiration date.

H. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther Fusion system verifies reagent levels.

I. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory’s standard quality control procedures.

J. Some reagents of this kit may be labeled with risk and safety symbols.

Note: Hazard Communication information for labeling of globally marketed products reflects the US and EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com.
Storage and Handling Requirements

A. The following table provides storage and handling requirements for the Panther Fusion Extraction Reagents-X.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Unopened Storage</th>
<th>On-board/Open Stability*</th>
<th>Opened Storage**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panther Fusion Capture Reagent-X</td>
<td>15 °C to 30 °C</td>
<td>30 days</td>
<td>15 °C to 30 °C</td>
</tr>
<tr>
<td>Panther Fusion Enhancer Reagent-X</td>
<td>15 °C to 30 °C</td>
<td>30 days</td>
<td>15 °C to 30 °C</td>
</tr>
</tbody>
</table>

*On board stability starts at the time the reagent is placed on the Panther Fusion system for the Panther Fusion FCR-X and FER-X.
**Working Panther Fusion Capture Reagent-X (Panther Fusion Capture Reagent-X that has been mixed with Internal Control-X on the Panther Fusion system) and Panther Fusion Enhancer Reagent-X are stable for 60 days when capped and stored at 15°C to 30°C. Do not refrigerate.

B. Discard any unused reagents that have surpassed their on board stability.

C. Avoid cross-contamination during reagent handling and storage.

D. Do not freeze reagents.
Sample Preparation

Definitions
- Specimens—Clinical material collected from a patient and placed in an appropriate transport system.
- Samples—Represents a more generic term to describe any material for testing on the Panther Fusion System including specimens, specimens transferred into a Panther Fusion compatible sample tube and controls.

Notes
- Refer to the Panther Fusion System Operator’s Manual for complete instructions on how to load samples onto the system.
- Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.
- Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over tubes.
- When testing frozen specimens, allow specimen to reach room temperature prior to processing.
- The following procedures are provided as guidance. Test specific sample preparation procedures should be developed and validated by the user.

Table 1 lists the minimum sample volumes needed based on the chosen tube type, number of extractions needed, and sample aspiration height.

Table 1. Minimum Sample Volumes

<table>
<thead>
<tr>
<th>Sample Tube</th>
<th>Part Number</th>
<th>Sample Aspiration Height</th>
<th>Cap</th>
<th>Required Dead Volume (µL)</th>
<th>Minimum Volume for a Single Extraction (µL)</th>
<th>Additional Volume for Each Additional Extraction (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aptima Specimen Aliquot Tubes (SATs)</td>
<td>503762</td>
<td>Low, Medium, High-Level Sensing</td>
<td>Pierceable, No Cap</td>
<td>200, 200, 200</td>
<td>550, 1150, 1300</td>
<td>350, 350, 350</td>
</tr>
<tr>
<td>100 Tubes (tapered)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transport Tube Polypropylene</td>
<td>401457</td>
<td>Low, Medium, High-Level Sensing</td>
<td>Pierceable, No Cap</td>
<td>900, 1300, 1300</td>
<td>1250, 1650, 1650</td>
<td>350, 350, 350</td>
</tr>
<tr>
<td>50 per bag</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The minimum volume may vary by sample type. Each sample type will need to be validated.
**Preparation of Working Diluents**

Specimens with high nucleic acid or cellular content will demonstrate greater extraction efficiency if diluted with a diluent (STM, UTM, PBS, or BTM) containing Open Access Diluent Additive.

1. Prepare working diluent stocks by pipetting 1.0 mL of Open Access Diluent Additive into 80 mL of STM, UTM, and BTM. To prepare a PBS working diluent stock, pipette 1.0 mL of Open Access Diluent Additive into 25 mL of PBS.
2. Mix by gently swirling the bottle or gently inverting. Do not vortex.
3. Label the bottle as Working Diluent-XXX where XXX = STM, UTM, PBS, or BTM.
4. Once prepared, the working diluents may be stored at room temperature (15°C to 30°C) for up to 30 days.

**Nasopharyngeal (NP) Swab in VTM and Nasal Swab in Liquid Amies Specimen Processing**

1. Transfer 500 μL of the NP or nasal swab specimen to an SLT.
2. Affix the provided penetrable cap or a new penetrable cap.

Alternatively,

1. Refer to Table 1 for the minimal volume required for the intended number of extractions for the sample tube being used.
2. Dilute the NP or nasal swab specimen in a 1:1.56 ratio with STM (e.g. combine 500 μL specimen with 780 μL of STM).
3. Affix the provided penetrable cap or a new penetrable cap.

**Lower Respiratory Tract (LRT) Specimen Processing**

1. Transfer 250 μL of the LRT specimen (avoid transferring mucus) and 250 μL of VTM to an SLT.
2. Affix the provided penetrable cap or a new penetrable cap.

Alternatively,

1. Refer to Table 1 for the minimal volume required for the intended number of extractions for the sample tube being used.
2. Dilute the LRT specimen in a 1:1 ratio with VTM (e.g. combine 250 μL of specimen with 250 μL of VTM).
3. Dilute the LRT/VTM mixture in a 1:1.56 ratio with STM (e.g. combine 500 μL of the mixture with 780 μL of STM).
4. Affix the provided penetrable cap or a new penetrable cap.

**EDTA Plasma and Serum Specimen Processing**

1. Refer to Table 1 for the minimal volume required for the intended number of extractions for the sample tube being used.
2. Dilute the plasma or serum specimen in a 1:0.2 ratio with Working Diluent-PBS (e.g. combine 500 μL of plasma or serum with 100 μL of Working Diluent-PBS).
3. Add Proteinase K to a final concentration of 0.5 mg/mL.
4. Incubate 30 minutes at room temperature (15°C to 30°C).
5. Affix the provided penetrable cap or a new penetrable cap.

**EDTA Whole Blood Specimen Processing**

1. Refer to Table 1 for the minimal volume required for the intended number of extractions for the sample tube being used.

   *Note: Processing of whole blood requires the use of the medium sample aspiration height.*

2. Dilute the whole blood specimen in a 1:2 ratio with Working Diluent-BTM (e.g. combine 400 µL of whole blood with 800 µL of Working Diluent-BTM).

3. Add Proteinase K to a final concentration of 1 mg/mL.

4. Incubate 30 minutes at room temperature (15°C to 30°C).

5. Affix the provided penetrable cap or a new penetrable cap.

**Stool Specimen Processing**

Prior to loading on the Panther Fusion System, stool samples must be transferred to the transport tube of an Aptima Multitest Swab Specimen Collection Kit.

1. Partially peel open the swab package. Remove the swab. Do not touch the soft tip or lay the swab down. Submerge the swab in the unformed or liquid stool specimen.

2. Uncap the transport tube containing 2.9 mL of STM. Place the swab in the transport tube and gently swirl the swab in the tube for 5 seconds to release material.

3. Carefully break the swab shaft at the score line against the side of the tube and discard the swab shaft.

4. Affix the provided penetrable cap or a new penetrable cap.

   *Note: To avoid the aspiration of flocculent material, stool specimen processing requires the use of medium sample aspiration height.*

**Urine Specimen Processing**

1. Refer to Table 1 for the minimal volume required for the intended number of extractions for the sample tube being used.

2. Dilute the urine specimen in a 1:1 ratio with Working Diluent-UTM (e.g. combine 300 µL of urine with 300 µL of Working Diluent-UTM).

3. Affix the provided penetrable cap or a new penetrable cap.

**Cerebrospinal Fluid (CSF) Specimen Processing**

1. Refer to Table 1 for the minimal volume required for the intended number of extractions for the sample tube being used.

2. Dilute the CSF specimen in a 1:1 ratio with Working Diluent-STM (e.g. combine 300 µL of CSF with 300 µL of Working Diluent-STM).

3. Affix the provided penetrable cap or a new penetrable cap.

**ThinPrep Specimen Processing**

1. Refer to Table 1 for the minimal volume required for the intended number of extractions for the sample tube being used.

2. Dilute the ThinPrep specimen in a 1:1 ratio with Working Diluent-STM (e.g. combine 300 µL of ThinPrep specimen with 300 µL of Working Diluent-STM).
3. Affix the provided penetrable cap or a new penetrable cap.

**Vaginal, Endocervical, Rectal, Throat, and Lesion Swab Specimen Processing**

*Note: Swab collection kits containing STM, VTM, or liquid Amies are acceptable for use.*

1. Refer to Table 1 for the minimal volume required for the intended number of extractions for the sample tube being used.
2. Dilute the swab specimen in a 1:1 ratio with Working Diluent-STM (e.g. combine 300 µL of swab specimen with 300 µL of Working Diluent-STM).
3. Affix the provided penetrable cap or a new penetrable cap.

**Lim or Carrot Broth Culture Specimen Processing**

1. Prior to testing on the Panther Fusion System, resuspend the culture specimen and transfer 1 mL of the specimen to the Aptima Specimen Transfer Tube containing 2.9 mL of Specimen Transport Medium (STM).
2. Affix the provided penetrable cap or a new penetrable cap.

**Panther Fusion System Test Procedure**

*Note: Refer to the Panther Fusion System Operator’s Manual for additional procedural information.*

**Work Area Preparation**

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared using the procedure described in step 1.

**Reagent Preparation**

1. Remove the bottles of IC-X, FCR-X and FER-X from storage.
2. Open the bottles of IC-X, FCR-X and FER-X, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion system.
3. Place the IC-X, FCR-X and FER-X bottles in the appropriate positions on the TCR carousel.
4. Close the TCR door.

*Note: The Panther Fusion system adds the IC-X to the FCR-X. After the IC-X is added to the FCR-X, it is referred to as wFCR-X (working FCR-X). If the FCR-X and FER-X are removed from the system, use new caps and immediately store according to the proper storage conditions.*

**Specimen Handling**

*Note: Prepare specimens per instructions in the Sample Preparation section before loading specimens onto the Panther Fusion system.*
Limitations

1. Do not vortex samples.
2. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

System Preparation

For instructions on setting up the Panther Fusion system including loading samples, reagents, assay cartridges and universal fluids, refer to the Panther Fusion System Operator’s Manual.

Limitations

A. Only use on Panther Fusion system by a trained professional.

B. Use of Panther Fusion Extraction Reagents-X for clinical specimen types not mentioned has not been validated.