# *Aptima® Real-Time General Purpose Reagents (RT-GPR) 100 Kit*

*100T RT-GPR*

For Laboratory Use.

**Materials Provided**

***Note:*** *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

| **Catalog Number: PRD-03914** | **100 reactions** |
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| **RT-GPR Refrigerated box (2° to 8°C):** | |
| Aptima Oligoless Amplification Reagent<br>*Nucleotides dried in buffered solution containing <5% bulking agent.* | 1x2.90mL<br>lyophilized |
| Aptima Enzyme Reagent<br>*Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.* | 1 x 2.26 mL<br>lyophilized |
| Aptima Oligoless Promoter Reagent<br>*Nucleotides dried in buffered solution containing < 5% bulking agent.* | 1 x 1.80 mL<br>lyophilized |
| **RT-GPR Non-refrigerated box (15° to 30°C):** | |
| Aptima Amplification Reconstitution Solution<br>*Aqueous solution containing preservatives.* | 1 x 7.20 mL |
| Aptima Enzyme Reconstitution Solution<br>*HEPES buffered solution containing a surfactant and glycerol.* | 1 x 5.80 mL |
| Aptima Promoter Reconstitution Solution<br>*Aqueous solution containing preservatives.* | 1 x 4.50 mL |
| Aptima Target Capture Reagent<br>*Buffered salt solution containing solid phase (<0.5 mg/ml) poly-deoxythymidine oligomers.* | 1 x 26.0 mL |
| Reconstitution Collars | 3 each |

**Product Description**

The Aptima Real-Time General Purpose Reagents (RT-GPR) are oligoless reagents to use in an *in vitro* nucleic acid amplification test (NAAT) for the qualitative or quantitative detection of targeted RNA using Transcription-Mediated Amplification (TMA®) and detection of the amplification products by fluorescent labeled probes (torches). The addition of Assay Specific Reagents (ASR) to the oligoless RT-GPR is required for target amplification and detection. Aptima RT-GPR may be used to facilitate the development of tests for the qualitative or quantitative detection of nucleic acid-based analytes.

**Principles of the Procedure**

The Aptima RT-GPR are designed to follow a standard protocol that involves three main steps, all taking place in a single tube: target capture, target amplification by transcription-mediated amplification (TMA), and detection of the amplification product (amplicon) by the fluorescent labeled probe (torch). During target capture, nucleic acids are isolated from specimens. The specimen is treated with a detergent to solubilize the organism, denature proteins, and release RNA. Capture oligonucleotides hybridize to highly conserved regions of the targeted RNA, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube.

Target amplification occurs via TMA, which is a transcription-mediated nucleic acid amplification method that utilizes two enzymes, MMLV (Moloney murine leukemia virus) reverse transcriptase and T7 RNA polymerase. The reverse transcriptase generates a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. Amplification of these specific regions is achieved using specific primers designed to amplify the desired target region.

Detection is achieved using a single-stranded nucleic acid torch that is present during the amplification of the target and that hybridizes specifically to the amplicon in real-time. Each torch has a fluorophore and a quencher. When the torch is not hybridized to the amplicon, the quencher is in close proximity of the fluorophore and suppresses the fluorescence. When the torch binds to the amplicon, the quencher is moved farther away from the fluorophore and the flourophore emits a signal at a specific wavelength when excited by a light source. As more torches hybridize to amplicons, a higher fluorescent signal is generated. The time (TTime) taken for the fluorescent signal to reach a specified threshold is proportional to the starting target concentration.

**Warnings and Precautions**

A. To reduce the risk of invalid results, carefully read the entire package insert prior to performing tests with the RT-GPR.
B. Only personnel adequately trained in the use of the Aptima RT-GPR and in handling potentially infectious materials should perform this procedure. If a spill occurs, immediately disinfect following appropriate site procedures.
C. Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash with water. If these fluids spill, dilute the spill with water before wiping dry. Please reference the appropriate Safety Data Sheet for additional information.

**Laboratory Related**

D. Use only supplied or specified disposable laboratory ware.
E. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

   **Note**: *As with any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are required.*

F. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Thoroughly clean and disinfect all work surfaces.
G. Dispose of all materials that have come in contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

**Storage and Handling Requirements**

A. The Aptima Promoter Buffer with oligonucleotides added is photosensitive. Store the solution protected from light.
B. The Target Capture Reagent is stable when stored at room temperature (15° to 30°C). Do not store at temperatures below 15°C.
C. DO NOT FREEZE THE REAGENTS.

**Reagent Reconstitution/Preparation**

A. Work Area Preparation
   1. Clean work surfaces where reagents are prepared. 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 then 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 are prepared. Use the procedure described above (step A.1).

B. Reconstitute the Aptima Real-Time General Purpose Amplification, Enzyme and Promoter Reagents
   1. Remove the lyophilized reagents (2ºC to 8ºC) and corresponding reconstitution solutions (15ºC to 30ºC) from storage.
   2. Before attaching the reconstitution collar, ensure that the reconstitution solution and lyophilized reagent are of the same reagent type (i.e. amplification reconstitution solution to amplification lyophilized reagent).
   3. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   4. Open the lyophilized reagent vial by removing the metallic seal and rubber stopper. Firmly insert the notched end of the reconstitution collar (black) into the vial (Figure 1, Step 1).
   5. Open the matching reconstitution solution bottle, and set the cap on a clean, covered work surface.
   6. Place the reconstitution solution bottle on a stable surface (i.e., bench). Then invert the lyophilized reagent vial over the reconstitution solution bottle and firmly attach the collar to the reconstitution solution bottle (Figure 1, Step 2).
   7. Slowly invert the assembled bottles (vial attached to solution bottle) to allow the solution to drain into the glass vial (Figure 1, Step 3).
   8. Pick up the assembled bottles and gently swirl. Avoid creating foam while swirling the bottle (Figure 1, Step 4).
   9. Wait for the lyophilized reagent to go into solution. After the lyophilized reagent has gone into solution, gently swirl to mix, then invert the assembled bottles again, tilting at a 45º angle to minimize foaming (Figure 1, Step 5). Slowly tilt the assembled bottles again to allow all of the solution to drain back into reconstitution solution bottle.
   10. Carefully remove the reconstitution collar and glass vial (Figure 1, Step 6).
   11. Recap the bottle. Record operator initials and reconstitution date on the label (Figure 1, Step 7).
   12. Discard the reconstitution collar and glass vial (Figure 1, Step 8).

   ***Warning:*** *Avoid creating foam when reconstituting reagents.*

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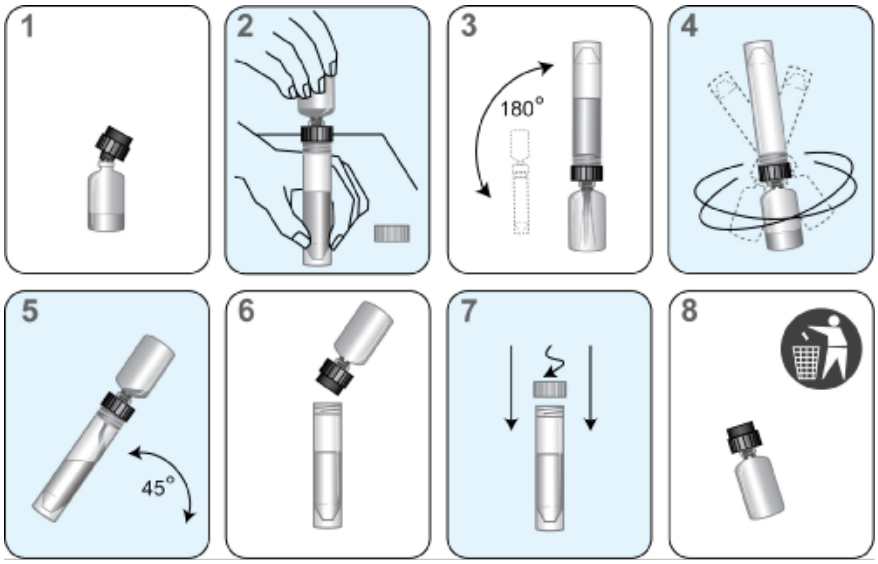


C. Reagent Preparation for Previously Prepared Reagents
   1. Remove the previously prepared reagents from storage (2ºC to 8ºC). Previously reconstituted Amplification, Enzyme, and Promoter Reagents must reach room temperature (15ºC to 30ºC) prior to the start of the assay.
   2. Invert the Amplification, Enzyme, and Promoter Reagents to mix thoroughly prior to loading on the system. Avoid creating excessive foam during inversion of reagents.
   3. Do not top off reagent bottles.

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