Quick-Start Protocol March 2017

AdnaTest BreastCancerDetect and AdnaTest ER/PR-Detect

AdnaTest BreastCancerDetect Box 2 (Box 2 of cat. no. 396012), containing the AdnaTest PrimerMixes and AdnaTest Positive Controls, and AdnaTest ER/PR-Detect (cat. no. 396062), containing the AdnaTest PrimerMixes and AdnaTest Positive Controls, must be stored separately at –30 to –15°C. To prevent possible contamination and repeated temperature changes, aliquot the primer mix. The components must not be used beyond the expiration date.

Further information

- *AdnaTest BreastCancerSelect, BreastCancerDetect and ER/PR-Detect Handbook*: **www.qiagen.com/HB-2453**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000 or **support.qiagen.com**

Protocol

1. Equilibrate AdnaTest Lysis/Binding Buffer and RNA Purification Buffers A and B to room temperature and place Tris-HCL Buffer on ice.
2. Wash 20 µl Oligo(dT)$_{25}$ Beads per sample twice with 20 µl AdnaTest Lysis/Binding Buffer per sample.
3. Add 20 µl washed Oligo(dT)$_{25}$ Beads to each sample.
4. Incubate for 10 min at room temperature under tilting and rotation at approximately 5 rpm.
5. Place the reaction tube in the AdnaMag-S and remove supernatant.
6. Wash beads with 2 x 100 µl RNA Purification Buffer A.

   Important: To avoid any loss of beads, rinse lid and tube wall thoroughly.
7. Resuspend beads in 100 µl RNA Purification Buffer B and transfer into a new 1.5 ml tube (provided).
8. Wash beads with 1 x 100 µl RNA Purification Buffer B.
9. Wash beads with 1 x 100 µl ice cold Tris-HCL Buffer.
10. Resuspend beads in 29.5 µl RNase-free water.
11. Incubate for 5 min at 50°C and place on ice for at least 2 min.

12. Continue with reverse transcription; see Table 1 and Table 2.

Table 1. Reverse transcription reaction setup

Component	Volume
RT master mix	
10x Buffer RT	4.0 µl
dNTP Mix (5 mM each dNTP)	4.0 µl
RNase inhibitor, 40 U/µl (Promega)	0.5 µl
Sensiscript Reverse Transcriptase	2.0 µl
Template RNA*	
mRNA/bead complex or RNase-free water	29.5 µl
Total volume	**40.0 µl**

* As RT control add 29.5 µl of RNase-free water instead of mRNA/bead complex. The volume of the mRNA/bead complex may vary slightly. Always use the total volume of this in the reverse transcription reaction.

Table 2. Reverse transcription program

Step	Time	Temperature
Reverse transcription	60 min	37°C
Denaturation	5 min	93°C
Cooling	∞	4°C

13. Continue with multiplex PCR for AdnaTest BreastDetect (Table 3 and Table 4) and/or AdnaTest ER/PR-Detect (Table 5 and Table 6) or store cDNA at –20°C for a maximum of 4 weeks.

Table 3. Preparation of the multiplex PCR (AdnaTest BreastDetect)

Component	Volume
Multiplex PCR master mix	
HotStarTaq Master Mix	25 µl
RNase-free water	13 µl
PrimerMix BreastDetect	4 µl
cDNA or RT control or Negative control (RNase-free water) or Positive Control (C+) each:	8 µl
Total volume	**50 µl**

Table 4. PCR cycling program (AdnaTest BreastDetect)

	Temperature	Time
Initial activation step	95°C	15 min
3-step cycling (35 cycles) Denaturation:	94°C	30 s
Annealing:	60°C	30 s
Extension:	72°C	60 s
Number of cycles:	35	
Final extension:	72°C	10 min
Cool-down:	4°C	∞

Table 5. Preparation of the multiplex PCR (AdnaTest ER/PR-Detect)

Component	Volume
Multiplex PCR master mix	
HotStarTaq Master Mix	25 µl
RNase-free water	13 µl
AdnaTest PrimerMix ER/PR-Detect	4 µl
cDNA or RT control or Negative control (RNase-free water) or AdnaTest Positive Control (C+) each:	8 µl
Total volume	**50 µl**

Table 6. PCR cycling program

	Temperature	Time
Initial activation step	95°C	15 min
3-step cycling (37 cycles) Denaturation:	94°C	30 s
Annealing:	60°C	30 s
Extension:	72°C	30 s
Number of cycles:	37	
Final extension:	72°C	5 min
Cool-down:	4°C	∞

14. For fragment analysis, use an Agilent® 2100 Analyzer or alternative analysis system.

    For evaluation of the results, please refer to the *AdnaTest BreastCancerSelect, BreastCancerDetect and ER/PR-Detect Handbook*.

IMPORTANT: If the protocol is not followed exactly, this may result in false-negative or false-positive results.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.