Quick-Start Protocol September 2017

AdnaTest LungCancerDetect

The AdnaTest RNA Reagent Box (Box 1 of cat. no. 396052) must be stored at 2–8°C. However, store the AdnaTest LungCancerDetect Box (Box 2 of cat. no. 396052), containing the AdnaTest PrimerMix EMT-2-Detect, PrimerMix LungDetect and positive controls, at –30 to –15°C. In order 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 LungCancer Handbook*: **www.qiagen.com/HB-2519**
- 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 (Table 3 and Table 4) and duplex PCR (Table 5 and Table 6) or store cDNA at –20°C for a maximum of 4 weeks.

Table 3. Preparation of the multiplex PCR (EMT-2-Detect)

Component	Volume
Multiplex PCR master mix	
HotStarTaq Master Mix	12.5 µl
RNase-free water	4.5 µl
AdnaTest PrimerMix EMT-2-Detect	4.0 µl
cDNA, RT control, negative control (RNase-free water) or AdnaTest Positive Control EMT-2, each:	4.0 µl
Total volume	**25.0 µl**

Table 4. PCR cycling program (EMT-2-Detect)

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

Table 5. Preparation of the duplex PCR (LungCancerDetect)

Component	Volume
Duplex PCR master mix	
HotStarTaq Master Mix	12.5 µl
RNase-free water	4.5 µl
PrimerMix LungDetect	4.0 µl
cDNA, RT control, negative control (RNase-free water) or AdnaTest Positive Control Lung each:	4.0 µl
Total volume	**25.0 µl**

Table 6. PCR cycling program (LungCancerDetect)

Step	Time	Temperature
Initial activation step	15 min	95°C
3-step cycling (35 cycles)		
Denaturation	30 s	94°C
Annealing	30 s	51°C
Extension	30 s	72°C
Final extension	5 min	72°C
Cooling	∞	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 LungCancer Handbook*.

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

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