

1. Description

SBT DiaDirect qPCR Solution has been developed so that clinical samples contained in Viral Transport Medium (VTM) can be used in qPCR studies without the need for any nucleic acid purification process. Thanks to its special formulation, it allows the viral pathogens contained in VTM to be broken down during the pre-denaturation step in the PCR reaction and nucleic acid to be released. While ensuring the stabilization of the nucleic acid released through the special chemicals contained in its content, it prevents non-specific binding during amplification and increases the amplification efficiency.

4. Direct qPCR Reaction Mix*

Content**	Quantity	Final Concentration
2X Master Mix	7,5 µL	1X
Primer Mix	2,5 µL	-
SBT DiaDirect	2,5 µL	-
Sample (VTM)	2,5 µL	-

* Calculate the 2X Master Mix, Primer Mix and SBT DiaDirect qPCR Solutions according to the number of samples, maintaining the proportions, and mix them in a separate tube.

Do not vortex. Make it homogeneous by slowly turning it upside down. Distribute the resulting mixture to the PCR wells in such a way that it is 12.5 µL.

** The content and quantity table may vary depending on the brand of the qPCR kit used. For optimal results, use SBT DiaDirect qPCR Solution with TÜSEB DiaKit branded qPCR kits. If you are using a different branded product, please contact our technical support department for SBT DiaDirect optimization.

5. SBT DiaDirect qPCR Solution Temperature Protocol

Temperature	Time	Cycle
95 °C	3 min.	1 cycle
95 °C	10 sec.	35 cycle
60 °C***	45 sec.	

*** The bonding-elongation temperature may differ depending on the qPCR kits used. Determine the connection-elongation Temperature according to the qPCR kit protocol you are using.

6. Interpretation of Graphical Results

- The auto threshold assigned by the PCR device should be used in the interpretation of the graphical results.
- **Linear** graphs under Threshold should be evaluated as negative.
- For graphs that remain under the threshold but are clearly in a **sigmoidal** structure, the reaction should be repeated.
- Graphs on which the device assigns a Ct value, but which are not in **sigmoidal** structure, should be evaluated negatively.
- On Multiplex qPCR kits, **linear** graphics may appear after the 33rd cycle. These graphics are usually the reflection of other paint channels. Such graphs should be evaluated as negative.

6. Limitations

- SBT DiaDirect qPCR Solution has been developed for DNA viruses. It is not suitable for use in RNA viruses.
- SBT DiaDirect qPCR Solution eliminates the extraction step. Due to this, the rate of protein contamination in PCR wells is increasing. This may reduce the sensitivity of the qPCR kit used by 1-2%.

