# TÜSEB DiaKit HighRisk HPV qPCR Diagnostic Kit Package Leaflet

## 1. Product Description

TÜSEB DiaKit HighRisk HPV qPCR Diagnostic Kit is manufactured according to EC directive 98/79/EC as an in vitro medical diagnostic kit. It is designed for professional use in clinical and research laboratories.

## 2. Method

This kit is based on the detection of HPV 16, 18, 45 types and 11 HPV types by real-time Polymerase Chain Reaction (qPCR) method. It is done by detecting the fluorescent radiation obtained as a result of amplification of specific regions of conserved E1/E2 genes.

**High-Risk** HPV types that the kit can detect: 31,33,35,39,51,52, 56,58,59,66,68 (**Primer Mix-2**).
The presence of high-risk HPV strains is indicated by the increase in fluorescence of the FAM channel. The kit makes it possible to detect HPV 16, 18 and 45 types in different channels at the same time. It detects the HPV16 species in the FAM fluorescent channel, the HPV 18 species in the HEX fluorescent channel, and the HPV 45 species in the ROX fluorescent channel (**Primer Mix-1**). The RNaseP gene is used as an internal control for DNA extraction quality control and possible qPCR inhibition control. Amplification of the RNaseP gene is detected in the Cy5 fluorescent channel for Primary Mix-1, in the HEX fluorescent channel for Primary Mix-2. The specially made molecular design takes advantage of the new suppressor (quencher) technology, which provides maximum sensitivity by minimizing nonspecific amplifications.

## 3. Technical Specifications

**Target Sequence:** E1/E2 genes

**Specificity:** Human Papillomavirus high-risk types 31,33,35,39,51,52,54,56,58,59,66,68 (PRIMER MIX-2)
Differentiation of 16,18,45 types (PRIMER MIX-1)

**Validated specimens:** Cervical, penis and vaginal swab, LBC

## 4. Storage Conditions

The kit should be transported at temperatures below -20 °C. As long as the storage temperature is maintained (between -15 °C and -25 °C), the kit can remain stable until the expiry date printed on the package. The components included in the kit should be used within a maximum of 5 repeated freeze/thaw cycles after the first use of a particular tube.

Cervical, penile and vaginal swab and LBC should be used for HPV detection. Swab samples in viral transport medium (VTM) can be stored at room temperature for up to 10 days. For long-term storage (6 months), it is necessary to keep the samples frozen at a temperature of -20 ± 5 ° C. For longer storage (3 years), it is necessary to keep the samples frozen at a temperature of -80 ± 5 °C. In order not to be affected by external conditions and temperature rises, after the receipt of the samples, it is recommended to transfer at a temperature of +2/+8 °C.

## 5. Contents of the Kit

| Kit contents | Quantity |
|---|---|
| | 1000 reactions |
| 2 x Master Mix | 6 x 1670 µL |
| Primer Mix-1 | 2 x 1250 µL |
| Primer Mix-2 | 2 x 1250 µL |
| Positive Control | 1 x 500 µL |
| Negative Control | 1 x 1000 µL |

***Table1.*** *Contents of the kit*

## 6. Nucleic Acid Isolation

It should be performed with commercially used isolation kits for nucleic acid isolation from certain clinical materials.

## 7. qPCR Reaction Setup Amplification Profile

The setup for 1 sample is as follows:

1. Add 5 µL of Master Mix into the Eppendorf tube.
2. Then add 2,5 µL of Primer Mix.
3. Distribute the resulting 7,5 µL reaction mixture into the strip or plate. **Repeat this process for each Primer Mix separately.**
4. Then add 2,5 µL of the isolation content obtained from clinical specimens onto this reaction mixture.
5. Care should be taken to ensure that the final volume in each well is 10 µL. As a result, two separate PCR reactions should be established in two separate wells for one DNA isolate.

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**SIGNIFICANT WARNING!**

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation.
After these steps, after making sure that the plate or strips are closed well, they should be loaded into the device. For each study, the Positive Control and Negative Control samples included in the kit should be included separately. Positive Control material does not involve any contamination danger to laboratory personnel. However, it is recommended that it be placed carefully so as not to contaminate other tubes and reaction mixes during installation. The manufacturer is not responsible for any deterioration of the kit due to misusage.

7. qPCR Reaction Setup and Amplification Profile is applicable to the extracted pure DNA. For working with SBT DiaDirect qPCR Solution, please review the title 8 Reaction Setup and Amplification Profile for SBT DiaDirect qPCR Solution.

| qPCR Protocol | | |
|---|---|---|
| **Cycles** | **Temperature (°C)** | **Time** |
| **1** | 95°C | 3 min. |
| **40** | 95°C | 5 sec. |
| | 60°C | 10 sec. |
| **Fluorescent Reading** | **FAM / HEX / ROX / CY5 (PRIMER MIX-1)**<br>**FAM / HEX (PRIMER MIX-2)** | |

**WARNING!!** Fluorescent channel names may differ depending on the devices used. Select fluorescent channels with the appropriate nanometer.

## 8. Reaction Setup and Amplification Profile for SBT DiaDirect qPCR Solution

SBT DiaDirect qPCR Solution has been developed that it can be used in qPCR studies without the need for any nucleic acid purification of clinical samples contained in Viral Transport Medium (VTM). 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 with its special formulaion.

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

Calculate according to the number of samples, keeping the proportions and mix the solutions in a separate tube which are 2X Master Mix, Primer Mix and SBT DiaDirect qPCR Solution. *Repeat this process separately for each Primary Mix included in the TÜSEB DiaKit HighRisk HPV qPCR Diagnostic Kit.* Do not vortex. Make it homogeneous by slowly turning it upside down. Distribute the obtained mixture to the PCR wells in such a way that it is 12.5 µL. Then add 2.5 µL of clinical samples (VTM) to this reaction mixture. It should be noted that the last volume in each well is 15 µL. As a result, two separate PCR reactions should be established in two separate wells for one clinical sample.

| qPCR Protocol | | |
|---|---|---|
| **Cycles** | **Temperature (°C)** | **Time** |
| **1** | 95°C | 3 min. |
| **35** | 95°C | 10 sec. |
| | 60°C | 45 sec. |
| **Fluorescent Reading** | **FAM / HEX / ROX / CY5 (PRIMER MIX-1)**<br>**FAM / HEX (PRIMER MIX-2)** | |

**In studies with SBT DiaDirect qPCR Solution;**
- The auto threshold assigned by the PCR device should be used in the interpretation of 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 that the device assigns a Ct value to but are not in sigmoidal structure should be evaluated as negative.
- Linear graphs may appear after the 33rd cycle. These graphics are usually the reflection of other dye channels. Such graphs should be evaluated as negative.
- SBT DiaDirect qPCR Solution eliminates the extraction step. For this reason, the rate of protein contamination in PCR wells is increasing. This may reduce the sensitivity of the qPCR kit used by 1-2%.

**For detailed information, please refer to the SBT DiaDirect qPCR Solution package leaflet.**

## 9. Interpretation of Results

| PRIMER MIX-1 (16,18,45 types) | | | | |
|---|---|---|---|---|
| **FAM** | **HEX** | **ROX** | **Cy5** | **EVALUATION** |
| - | - | - | + | Hpv 16,18,45 Neg. |
| + | - | - | + | Hpv 16 Pos. |
| - | + | - | + | Hpv 18 Pos. |
| - | - | + | + | Hpv 45 Pos. |
| - | - | - | - | Invalid |
| Internal control (Cy5) should always be **positive** when other channels are **negative**. Otherwise, the test should be repeated. If there is a **positive** result in any of the other channels and the internal control is **negative**, the result is considered valid. | | | | |

| PRIMER MIX-2 (High Risk Types: 31,33,35,39,51,52,54,56,58,59,66,68) | | |
|---|---|---|
| **FAM** | **HEX** | **EVALUATION** |
| - | + | High Risk Types Neg. |
| + | + | High Risk Types Pos. |
| - | - | Invalid |
| !! Positive value in the Fam channel means positive for at least one of the HPV types listed above. When the FAM channel is negative, the internal control (HEX) should always be positive. Otherwise, the test should be repeated. If there is a positive result in the FAM channel and the internal control is negative, the result is considered valid. | | |

**WARNING**

A single valid Package Leaflet for the kit is included in the package or will be requested from the manufacturer for a particular batch. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit does not contain any dangerous, infectious or toxic components that would be subject to special safety regulations, and the packaging materials are made of paper and polypropylene. Please get in contact if you have any questions.