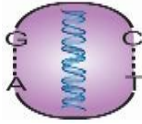


TELSA-Dx

**Instructions
for Use.
Multiplex
Real-Time
TEL-PCR-HPV-
16-18.**



TELSA-Dx

Instructions for Use.

1. Application

HPV is a sexually transmitted infection, and the viral DNA infection could be classified into low and high-risk genotypes. High risk genotypes like HPV-16 and 18 causes 80% of cervical cancers in women, and 90% HPV-related cancers in men¹. While low risk genotypes like HPV-6 and 11 causes 95% of genital warts; not all HPV infections causes cancer¹. TELSADx has in stock both single-plex and multiplex TEL-HPV-PCR kits for 20 HPV genotypes: contact PCR@TELSA-Dx.com for details.

Real-Time TEL-PCR-HPV-16-18 is a multiplex PCR reagent system for the qualitative detection of Human Papilloma Virus (HPV-16 and HPV-18 respectively). The analytical sensitivity of the assay is 10 DNA copies per reaction for HPV-16 and HPV-18 respectively. While 100% analytical specificity was established for both genotypes.

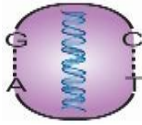
Detection of HPV from cervical swab gives a higher sensitivity when compared with vaginal swab, but vaginal swab is more sensitive than a Pap smear and no cervical screening². The detection of HPV DNA can be combined with our simple and easy to use TEL-DNA extraction kit.

2.0. TEL-PCR-HPV-16-18 Kit Components

Component	Number of vials	Volume (µL/vial)	Number of total tests/vials
TEL-HPV-16-18 Master Mix	1	630	50
TEL-HPV-16-18 Primer Mix	1	120	50
TEL-HPV-16-18 Positive Control	1	250	50
TEL-HPV-16-18 Negative Control	1	250	50

The Positive Control contains HPV cloned target, and it is non-infectious. **Avoid repeated freezing and thawing of this control.**

TEL-Negative Control is also non-infectious.



TELSA-Dx

3.0. Storage of Kit Components

TEL-PCR-HPV-16-18 kit should be stored at -20°C on arrival. If tubes have been compromised on arrival, contact PCR@TELSA-Dx.com immediately. All components should be stored according to the instruction on the labels. Where possible, avoid repeated thawing and freezing of Master Mix, Primer mix, and positive control. It is advisable to not freeze and thaw more than twice, because 100% efficiency of the kits might be affected. The reagents should be frozen in aliquots if they are to be used intermittently. Storage between 2°C and 8°C should not be more than two hours to maintain 100% efficiency of the kits. Do protect TEL-HPV-16-18 Master Mix from light by using aluminium foil as an example.

4.0. Real time TEL-PCR-HPV-16-18 kit description

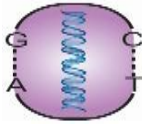
TEL-PCR-HPV-16-18 is a reagent system based on our proprietary PCR reagent system and successful reaction occurs in a single tube on addition of Primer mix and extracted DNA. The kit contains all the required components for a successful Real-Time PCR detection of HPV-16 or HPV-18 or both genotypes in one reaction setup.

5.0. Real-Time PCR equipment's

TEL-PCR reagent system could be used with any opened Real-Time PCR detection system. Always ensure that all the equipment's used have been installed, calibrated, checked, and maintained according to the manufacturer's instructions and recommendations.

6.0. Real time TEL-PCR reaction set-up

- To set up 20 µl reaction, refer to the table below.
- It is advisable to prepare your master-mix based on the number of samples to be tested. Assuming you have 2 samples, multiply 2 by the required reagent (master-mix) volume indicated below (for example, 12.6 x 2 = 25.2 µl) and dispense it into your master-mix tube (not provided).



TELSA-Dx

Repeat the same process for primer-mix (for example, $2.4 \times 2 = 4.8 \mu\text{l}$) and dispense into same master-mix tube. Mix properly and centrifuge briefly.

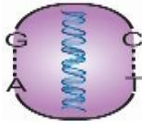
- Aliquot $15 \mu\text{l}$ of the mixed master-mix into each required well of an appropriate optical 96-well reaction plate or tube.
- Add $5 \mu\text{l}$ of the template: extracted DNA or $5 \mu\text{l}$ of the controls (positive or negative).

Reaction set-up for one test	
Master-mix	$12.6 \mu\text{l}$
Primer Mix	$2.4 \mu\text{l}$
DNA or control	$5 \mu\text{l}$
Final volume	$20 \mu\text{l}$

- Ensure you run one positive and negative controls per reaction set-up.
- Thoroughly mix the primer-mix and DNA or controls with the Master-Mix by pipetting up and down.
- Cover up the 96-well reaction plate with optical adhesive film and the reaction tubes with appropriate optical lids.
- If possible, centrifuge the 96-well reaction plate/tube in a centrifuge with a microtiter plate rotor (if it is a reaction plate) for 30 seconds at approximately $1000 \times g$ (~ 3000 rpm).

7.0. Temperature profile and FAM/HEX acquisition

	Stage	Number of cycles	Acquisition	Temperature ($^{\circ}\text{C}$)	Time (min:sec)
Denaturation	Hold	1	No	95	1:00
Amplification	Cycling	45	No	95	00:10
			Yes	60	00:30



TELSA-Dx

8.0. Interpretation of results; qualitative analysis

Detection channel	Result interpretation
FAM/HEX	
FAM: HPV-16 positive reaction (≤ 45.00 Cq)	HPV-16 target detected
HEX: HPV-18 positive reaction (≤ 45.00 Cq)	HPV-18 target detected
*Negative reaction (0.00 Cq)	HPV-16 (FAM) or HPV-18 (HEX) target not detected. The sample does not contain detectable HPV-16 or HPV-18.

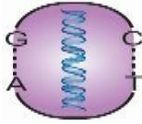
*Repeat the reaction set-up if negative control is falsely positive.

This could be due to cross-contamination or contamination issues with your set-up. If it is still falsely positive, use a new aliquot of master-mix, primer-mix, and controls. Refer to section 9.0 for good practices within PCR working space.

9.0. Good laboratory practices for PCR

Adhere strictly to the following when preparing samples for PCR or real-time PCR:

- A. Wear clean gloves and a clean laboratory coat.
 - Do not wear the same gloves and laboratory coat used previously for handling amplified products or preparing samples.
- B. Change gloves if you suspect that they are contaminated.
- C. Change your NTC or/and negative control or Master Mix if you suspect template contamination.
- D. Maintain separate areas and dedicated equipment and supplies for sample preparation and reaction setup, and amplification and analysis of amplified products.
- E. Do not bring amplified products into the reaction setup area.



TELSA-Dx

- F. Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- G. Keep reactions and components capped as much as possible.
- H. Use a positive-displacement pipette or aerosol resistant barrier pipette tips.
- I. Clean laboratory benches and equipment periodically with freshly prepared 10% bleach solution or DNA decontamination solution.

References:

1. Australian Government, Department of Health. Human papillomavirus. <https://www.health.gov.au/health-topics/hpv-human-papillomavirus>
2. Royal Australian College of General Practitioners. Self-collection of HPV samples: A guide for GPs. <https://www1.racgp.org.au/newsgp/clinical/self-collection-of-hpv-samples-a-guide-for-gps>

For Professional Use or IVD.

©TELSA-Dx.com. 2023. All rights reserved.