1. Intended Use of the Kit and Test Principle

The kit is used for rapid and accurate diagnosis of the viral agents listed in Table 1a in clinical samples. The kit is applied to nucleic acid isolates obtained from cervical, penile and vaginal swabs. Rapid diagnosis with the kit is performed by real-time PCR (qPCR) targeting genomic DNA regions specific to the target agent. Diagnosis with the kit can be performed in less than 1 hour*. The kit is applied by healthcare providers to cervical, penile or vaginal swabs taken from individuals with suspected disease.

***With Bio-Rad CFX96 Touch**

2. Kit Contents

Shelf life: 12 months; expiration date on the box see below. Each reagent, tube stored at storage temperature can be used until the expiration date indicated on the kit. The expiration date of the kit is determined by the expiration date of the reagents.

2.1. Kit Storage Temperature

The kit is stable until the expiry date printed on the packaging as long as the storage temperature (between -15°C and -25°C) is maintained.

Table 1a. Kit content

Content/Purpose of Use	Channel	Content	Quantitay(10 µL Reaction)	Unit Reaction Consumption
			250 Reaction	
DNA polymerase, dNTP mix, reaction buffer and ribonuclease inhibitor	-	**2X Master Mix**	4 x 1250 µL	5 µL
HPV 39	FAM	**Primer Mix 1**	1 x 625 µL	2,5 µL
HPV 52	HEX			
HPV 35	ROX			
IC; Internal Control, (Human RNase P gene)	CY5			
HPV 33	FAM	**Primer Mix 2**	1 x 625 µL	2,5 µL
HPV 6/11	HEX			
HPV 68	ROX			
IC; Internal Control, (Human RNase P gene)	CY5			
HPV 51	FAM	**Primer Mix 3**	1 x 625 µL	2,5 µL
HPV 56	HEX			
HPV 31	ROX			
IC; Internal Control, (Human RNase P gene)	CY5			
HPV 66	FAM	**Primer Mix 4**	1 x 625 µL	2,5 µL
HPV 58	HEX			
HPV 59	ROX			
IC; Internal Control, (Human RNase P gene)	CY5			

Table 1b. Kit content-Controls

Storage Temperature: 2-8°C; Transfer Temperature: 2-8°C Store at -20°C if components are frozen. Store at 2-8°C after initial thawing.			
Negative Control (Nuclease Free Water) Test at each operation to check for contamination. **Separate reaction for each primer mix	NTC	1 x 1000 µL	2,5 µL
Positive Control Plasmid containing target gene regions Test at each run to check reagent stability. **Separate reaction for each primer mix	PC	1x 500 µL	2,5 µL

Table 1c. Devices and equipment to be provided by the user

Devices and equipment to be provided bythe user	
1. Real-Time PCR Instrument: 4 channels, Ramp rate ≥3 °C/sec. **2.** 1-10 µL micropipette and compatible pipette tip (DNase and Rnazfree) **3.** Spin Centrifuge: min.3000 rpm (compatible with 8-strip and2 mL microcentrifuge tube) **4.** Vortex	**5.** UV cabinet for PCR setup **6.** Cold Tube stand (microcentrifuge tubes and PCRfor tubes/strips) **7.** Disposable, powder-free, nitrile gloves.

3. Validated qPCR Instruments

The SBT DiaPatogen CerviX12 HPV Genotyping Kit was validated with the instruments given in **Table 2**.

Table 2. Validated qPCR instrument specific plastic consumables

CFX96 Touch*	***Rotor-Gene® Q***
Cat#: 1845098; CFX Qualification plate andfilm(96 wells)	Cat#: 981103; Qiagen Strip Tube 0.1 mL (4 well)
Cat#: BS2001 Ultra-optical semi-skirted qPCR plateve movie (96 wells)	
Cat#: TLS0851; White Strip (8 wells)	

***Use only white plate/white strip with Bio-Rad CFX96 qPCR instrument!**

4. Collection, Storage and Transfer of Nucleic Acid Extracts

Swab samples should be collected using Dacron or Polyester swabs. Other sample types should be transferred in sterile containers. Viral Transport Medium (VTM) (Preparation of viral transport medium, Center for Disease Control and Prevention, SOP #: DSR-052-01) should be used. Samples should be stored and transported at 2-8°C until they arrive at the laboratory. Swab samples should be transferred within 5 days, other sample types within 2 days. If a delay in shipment is expected, samples should be frozen at -70°C and shipped on dry ice. It is important that samples are not subjected to repeated freezing and thawing. **Samples arriving at the laboratory in VTM should be subjected to nucleic acid extraction.**

5. Warnings

1. The kit can be used from nucleic acid sources and qPCRamplicons keep it away from the water.
2. You may use kit components with different lot numbers or withthe same name. Do not mix with chemicals from different manufacturers.
3. Keep the master stock reagents in cold block during PCRsetup.
4. If possible, set up the PCR in cold block.
5. Gently mix the kit components before use.
6. Pipetting qPCR mixtures and template nucleic acidsuse separate micropipettes.
7. Keep the template nucleic acid and positive control tubes closed at all times except during liquid transfers.
8. The room, benches and devices where the test is performed can be wiped clean clean the surfaces regularly with 10% NaClO.

6. Analytical Performance

Pre-clinical optimization and validation of the SBT DiaPatogen CerviX12 HPV Genotyping Kit was performed using sequences of positive control clones (K02718, X05015, J04353, M12732, X74477, M62849, X74479, M62877, X74481, U37488, X74483, D90400, X77858, U31794, X67161) from the Karolinska Institute International Human Papillomavirus Reference Center. Reproducibility, sensitivity, specificity, linearity and sensitivity parameters were examined and optimized in these studies

7. qPCR Application Protocol

Consider the following information before starting the analysis:

1. The kit has only been validated for a volume of template nucleic acid that is 25% of the total qPCR volume.
2. The kit should not be used with real time PCR instruments without periodic maintenance records.
3. Do not use a qPCR plate/strip that is not validated with the kit!

4. Program the qPCR instrument as indicated below and add the reagents to the qPCR tubes in the order indicated below, seal the tubes, place them in the qPCR instrument and start the run (Table 3).

Table 3. Reaction setup and qPCR program details.

Reaction Setup		**qPCR Program**		
Content	**Volume**	**Number of Cycle**	**Temperature**	**Duration**
2X Master Mix	5 µL	1	95°C	3 dk
Target Primer Mix	2,5 µL	39	95°C	10 sn
Samlpe Nucleic Acid	2,5 µL		60 °C	15 sn
Total Reaction Volume	10 µL	**Reading FAM / HEX / ROX / CY5**		

8. Interpretation of Test Results

The shape of the amplification curves should be examined. If a sample is assigned a Cq value by the instrument software and the curve is sigmoidal, the Cq value can be used in the final evaluation. **Non-sigmoidal curves should be recorded as negative. If a sample has been assigned a Cq value, but the curve is not sigmoidal, the result should be recorded as negative.**

For samples with a suspicious sigmoidal curve pattern below threshold in the channel of the targets, the Cq value of the IC should be examined. If IC Cq≤34, the sample should be reported as negative. If Cq>34, the test should be repeated after freezing and thawing the sample. If the problem persists after freeze-thaw, a new sample should be requested.

Table 4. Expected performance of kit controls.

Control Type	**Name**	**Control Objextive**	**Expected Conclusion**	
Addition of NTC	NTC	Contamination control	**No Cq = Valid**	
Do not add template	NRC	Reagent contamination control	**No Cq = Valid**	
PC addition	PC	Positive reactive control	**Cq ≤ 36.0 = Valid**	
Human RNaseP	IC	Control of sampling, DNA integrity, nucleic acid extraction, and inhibition of qPCR	**Cq≤34.0 = Valid**	**If IC Cq ≥34.0, but target Cq≤36.0, IC valid**

If any control fails to operate as described in Table 4, the run is considered invalid and the test is repeated.

1. Invalid PC: Contact the manufacturer, replace the reagents and repeat the reaction.
2. Invalid NRC: Contact the manufacturer, renew the reagents and repeat the reaction.
3. Invalid NTC: Repeat the analysis observing the "**Warnings**" section.

If all checks are valid, proceed to the interpretation of the results.

- If the Cq value of the gene targets is **≤ 36**, conclude as **positive**.
- If the Cq value of the gene targets is **> 36**, conclude as **negative**.

4. Limitations

- SBT DiaPatogen CerviX12 HPV Genotyping Kit performance was determined in cervical, penile and vaginal swab samples.
- Mutations in the target regions of the kit may affect primer and/or probe binding and result in the presence of virus not being detected.
- Errors made during sample collection, transportation or handling can lead to false negative results.
- Inhibitors or other interfering factors may cause a false negative result. Insufficient number of target organisms in the sample false-negative results may also occur.