Shanghai Promega

CE 0086 IVD

For Professional Use Only

ProDx™ HSV-1/2 DNA qPCR Detection Kit

Instruction for Use

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1. INTENDED USE

The *ProDx*™ HSV-1/2 DNA qPCR detection Kit is a Taqman® fluorescent probe based Real-Time Polymerase Chain Reaction assay. This product is intended for *in vitro* diagnostics use. It is designed to detect herpes simplex virus 1 and 2 viral DNA from cervical and urethral swabs. This assay does not quantify viral loads. Diagnostic results obtained using this product must be interpreted in conjunction with other clinical or laboratory data.

2. INTRODUCTION

Herpes simplex virus (HSV) is a common human pathogen worldwide. HSV transmission can occur due to direct contact with infected secretions from either a symptomatic or an asymptomatic patient. There are 2 serotypes of Herpes simplex virus: HSV-1 and HSV-2. HSV-1 is generally associated with infection in the tongue, mouth, lips, pharynx and eyes. HSV-1 is transmitted through oral secretion or sores on the skin. It can be spread through kissing, sharing toothbrushes and eating utensils. HSV-2 is primarily associated with genital and neonatal infection. HSV-2 is mostly transmitted through sexual contact with infected person.

HSV-2 infection is the leading cause for genital ulcers for sexually active adults in many countries. Genital ulcers have been associated with an increased risk for HIV infection. In many cases, genital herpes are transmitted by persons who are not self ware of infection or exhibit little or atypical symptoms. The Centers for Disease Control and Prevention (CDC) states that counseling is an important aspect of managing patients who have genital herpes.

3. PRINCIPLE OF PCR DETECTION

ProDx™ HSV-1/2 DNA qPCR Detection Kit is a three-color multiplex fluorescent probe based Taqman® Real-Time PCR assay system. The assay can specifically detect Herpes simplex virus (HSV)-1 and HSV-2 viral DNA and an endogenous internal control (IC) DNA that is a highly conserved region of human β-globin gene (HBB) in the same reaction. The internal control is used to control the whole assay workflow efficiency from specimen collection, DNA extraction, PCR amplification to data analysis. The amplification system also includes dUTP and Uracil N-Glycosylase (UNG) to minimize amplification product contamination. The amplified HSV1, HSV2, and IC DNA fragments are detected by JOE, FAM and ROX channels, using Applied Biosystems 7500 Real-Time PCR System.

4. PRODUCT CONTENTS

Reagent	Part No.	Quantity	Volume/tube
HSV-1/2 DNA Extraction Solution	CA736	1 tube	3 ml
HSV-1/2 PCR Reaction Mix	CA737	2 tubes	1.1 ml
HSV-1/2 Enzyme Mix	CA738	1 tube	0.03 ml
HSV-1/2 Positive Control	CA739	1 tube	0.3 ml
HSV-1/2 Negative Control	CA740	1 tube	0.5 ml

Each system contains sufficient reagents to perform 48 reactions.

Reagents:

	Component	Ingredient
ProDx™ HSV-1/2 DNA qPCR Detection Kit	HSV-1/2 DNA Extraction Solution	5% Chelex-100,EDTA
	HSV-1/2 PCR Reaction Mix	Tris-HCl, EDTA, Potassium chloride, <1% proprietary PCR stabilizer <5.5mM Magnesium chloride <0.55mM dATP, dCTP, dGTP, dUTP <50 mM primers and probes
	HSV-1/2 Enzyme Mix	<4 % GoTaq DNA Polymerase and Uracil-DNA Glycocasylase, Glycerol
	HSV-1/2 Positive Control	non-infectious plasmid DNA
	HSV-1/2 Negative Control	H_2O

5. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

- Biological Safety Cabinet
- Benchtop micro centrifuge
- Sterile saline (0.9% NaCl)
- Applied Biosystems 7500 Real-Time PCR System
- 96-well reaction plates or tube strips or individual tubes suitable for 7500
- Micropipettes with a minimal accuracy range between 1–10µl, 10–100 µl and 100–1000µl, and sterile, nuclease-free aerosol-barrier micropipettor tips
- Vortex mixer
- Disposable powder free latex gloves
- Biohazard waste container
- Dry heater block

6. STORAGE AND PRODUCT STABILITY

- All reagents should be stored at –20°C.
- The reagents are stable till the expiration date as marked on the packaging label when stored at the recommended condition.
- Avoid multiple freeze-thaw cycles (<5 times).
- Avoid direct exposure of kit components to light, heat or humidity.
- Thaw reagents at room temperature (15°C–25°C) prior to use. Once the reagents have been thawed, vortex and centrifuge the tubes briefly to ensure the reagents to be mixed uniformly.

7. WARNINGS AND PRECAUTION

- For *in vitro* diagnostic use only.
- Please read this manual carefully before beginning the experiment.
- The product should be only used by trained labor personnel with PCR amplification and other required lab skill.
- Apply Universal Precautions for health care worker safety. All patient specimens should be considered as potentially infectious and handled properly. Wear personal protective equipment when handling specimens and reagents according to Good Laboratory Practices for safety and cross-contamination controls. Specimens and controls should be prepared in Biological Safety Cabinet.
- Clean and disinfect any accidental spills with disinfectant such as 0.5% sodium hypochlorite, followed by wiping down the surface with 70% ethanol.
- The assay workflow must follow a definite unidirectional process: starting from reagent preparation in the Reagent Preparation Area, moving to Specimen Preparation Area for nucleic acid extraction and PCR plate assembly, and then Amplification Area for PCR reaction. Do not return specimens, equipment and reagents back to the previous step. Lab personnel should follow proper anti-contamination procedures when moving between areas.
- Use separate lab supplies and equipment in dedicated areas for reagent preparation and sample/ specimen preparation. Do not back track samples, reagents, and exchange lab equipment between different areas.
- To prevent contamination, use aerosol barrier pipette tips for pipetting.
- Do not use a kit if it is damaged or expired.
- Do not mix or interchange reagents from different kit lots or from other manufacturers.
- Do not interchange reagent tube / bottle caps.
- Dispose of used, unused kit reagents and human specimens according to local, state and federal regulations.

8. SAMPLING AND HANDLING

Specimen Type:

The swab specimen is collected according to standard clinical proved procedure. Only the following sample types are validated for this assay.

- Male: urethral swabs
- Female: cervical swabs

Storage and Transportation of Specimens:

The specimens should be extracted immediately for the best result. The specimens can be kept at 2–8°C up to 24 hours or at –20°C up to 1 month. Specimens can be transported in sealed bubble wrap on ice or dry ice. Frozen specimens should be thawed at room temperature (15°C–25°C) before the assay. Avoid repeated freeze-thaw cycles.

Note: The patient should be advised not to urinate for at least 2 hours and should not have sex for 24 hours prior to specimen collection.

9. PROTOCOL

9.1 Reagent Preparation (Reagent reparation Area)

1. Thaw all the assay reagents at room temperature (15°C–25°C) before use. Vortex briefly and spin in down to ensure the reagent mixed uniformly.
2. For each experiment set, prepare a PCR mix sufficient for n + 2 PCR reactions (n specimens +1 for HSV-1/2 Positive Control +1 for HSV-1/2 Negative Control). The total PCR reaction volume is 50μl, including 45μl PCR mix and 5μl DNA sample. See table below:

Reagent	Volume Per PCR Reaction	Volume for n+2 PCR Reaction
HSV-1/2 PCR Reaction Mix	44.4μl	44.4μl×(_+2)=_μl
HSV-1/2 Enzyme Mix	0.6μl	0.6μl×(_+2)=_μl
PCR mix volume	45μl	45μl×(_+2)=_μl
Template DNA	5μl	—
Total PCR volume	50μl	—

3. Pipette 45μl PCR mix to real-time PCR reaction plate or tubes. Seal the plate and close the tubes and move the plate or tubes to Specimen Preparation Area. The plate or tubes should be kept away from direct light.

9.2 Specimen Preparation (Specimen Preparation Area)

1. Add 1.0ml sterile saline to the urethral or cervical epithelial swabs container and vortex vigorously for 5 seconds. Remove the swab.
2. Transfer 500μl of the swab saline to a new tube and centrifuge at 12000 rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
3. Wash the pellet once by adding 1.0ml saline and resuspend the pellet by vortexing for 5 seconds. Centrifuge at 12,000 rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
4. Add 50μl fully resuspended HSV-1/2 DNA Extraction Solution, close the tube then suspend the pellet by vortex vigorously for 5 seconds. Spin down all the liquid by a quick centrifugation.
5. Heat the tube with HSV-1/2 DNA Extraction Solution at 100°C for 10 minutes. Close the tube lid tightly to prevent evaporation or cross contamination.
6. Centrifuge the tube at 12,000 rpm for 5 minutes. The supernatant contains the extracted DNA sample for PCR Amplification in next step.

9.3 PCR Reaction Preparation (Specimen Preparation Area)

1. Add 5μl DNA samples, 5μl HSV-1/2 Positive Control and 5μl HSV-1/2 Negative Control to different reaction wells or tubes which contained 45μl PCR mix. Immediately seal the plate or close the tubes to avoid contamination. Spin briefly to collect all the liquids to the bottom of PCR wells or tubes.
2. Perform PCR without unnecessary delay after assembling the PCR reactions.

9.4 Amplification Specification (Amplification Area)

The product is optimized for use with Applied Biosystems 7500 Real-Time PCR System. Please see 7500/7500 Fast Maintenance Guide and Absolute Quantitation Using Standard Curve Getting Started Guide from Applied Biosystems for instrument maintenance, calibration, and operation guidance. Setup the following cycling protocol in the instrument:

Step	Temperature(°C)	Time (sec)	Fluorescence detection	Repeats
1	37	300	-	1
2	95	300	-	1
3	95	10	-	45
4	60	50	JOE, FAM, ROX	

Note: Adjust the passive reference setting to None.

10. DATA ANALYSIS

A positive reaction is detected by accumulation of a fluorescent signal measured by cycle threshold (Ct), which is defined as the number of cycles required for the fluorescent signal to pass the threshold. During data analysis, the baseline is set automatically between 3–15 cycles. Passive reference is set to None. The threshold should be set above the amplification curve for the Negative Control (background noise line), so that the negative control Ct is shown as Undet. The Ct values are automatically generated by the instrument software.

The amplification result is valid when all the following criteria are met:

1. Negative Control generates no signal (Ct = 45 or Undet) in all channels (ROX, JOE and FAM).

2. Positive Control generates positive signals (Ct < 32) in channel JOE and FAM.
3. The unknown specimen is scored based on Ct value for positive or negative based on the table below in each fluorescent channel:

Channel	Ct value	Results
ROX (IC)	Ct = 45 or Undet	Negative (-)
	Ct < 45 with typical amplification curve	Positive (+)
JOE (HSV-1) FAM(HSV-2)	Ct = 45 or Undet	Negative (-)
	37 ≤ Ct < 45 with typical amplification curve	Ambiguous, repeat the test, if Ct < 45 again, it is positive.
	Ct < 37 with typical amplification curve	Positive (+)

The unknown specimen HSV-1/2 phenotype is interpreted based on the following table:

Channel ROX (Internal control HBB)	**Channel JOE** (HSV-1)	**Channel FAM** (HSV-2)	**Results**
+	-	-	HSV1 and HSV2 both negative
+or-	+	-	HSV1 positive, HSV2 negative
+or-	-	+	HSV2 positive, HSV1 negative
+or-	+	+	HSV1 and HSV2 both positive
-	-	-	PCR failure, which is maybe caused by inhibitors from DNA extraction. Repeat the PCR experiment with samples diluted by 10 or 100 folds. Or Repeat the specimen collection.

Note: "+" is Positive; "-" is Negative.

11. LIMITATIONS

1. The system is designed for use by trained professionals. The complete assay process should be validated for clinical data interpretation.
2. Improper specimen collection, storage or handling may reduce template concentration or increase contamination risk which can generate a false negative result or false positive result.
3. A false negative result may occur if an excess of DNA/RNA template is present in the reaction. Repeat the test with 1:2, 1:10 or 1:100 dilutions of the test samples.
4. Target sequence variations may lead to false negative result.
5. Use good laboratory practice to minimize the cross contamination between samples and exogenous DNA. The instructions in this document must be strictly followed. Use good laboratory practice to minimize the cross contamination between samples and exogenous DNA. The instructions in this document must be strictly followed.

12. PERFORMANCE SPECIFICATIONS

12.1 Analytical Sensitivity

ProDx™ HSV-1/2 DNA qPCR Detection Kit was tested with the serial dilution of positive controls. The limit of detection (analytical sensitivity) for this kit is equivalent to 5 copies/μl for HSV-1 and HSV-2 plasmid DNA.

12.2 Analytical Specificity

ProDx™ HSV-1/2 DNA qPCR Detection Kit was able to detect HSV-1/2 from multiple positive specimens and reference materials. The kit was tested negative against CT, NG, UU, HPV and 11 negative reference microorganisms commonly isolated from the urogenital tract. The reference microorganisms list is recommended and offered by China National Institutes for Food and Drug Control.

List of negative microorganisms commonly isolated from the urogenital tract:

Latin Name	Strain Number	Lot
Candida albicans	CMCC(B)98001	98001-2a1
Enterococcus faecalis	140623	140623-200708
Clostridium sporogenes	CMCC(B)64941	64941-2A4

Bacillus pumilus	CMCC (B) 63202	63202-4a2
Streptococcus hemolytis-β	CMCC (B) 32210	32210-4a
Serratia marcescens subsp marcescens	CMCC(B)41002	41002-14
Salmonella enterica subsp enterica	CMCC(B)50041	50041-16
Pseudomonas aeruginosa	CMCC (B) 10211	10211-8
Staphylococcus aureus subsp.aureus.	CMCC (B) 26112	26112-10
Micrococcus luteus	CMCC (B) 28001	28001-8a1
Escherichia coli	CMCC(B)44102	44102-3a9
	CMCC (B) 44113	44113-11

12.3 Assay Precision

The intra-batch reproducibility variation coefficients are less than 5%, determined by repeat testing with reference sample group for more than 10 times. The reference sample group includes high and low titer specimens.

The inter-batched reproducibility tests showed assay variation coefficients are less than 5% when the assays were conducted by 3 different operators, each repeated tests 10 times with reference sample group.

12.4 Clinical Data and Result

The clinical evaluation was conducted with 665 unknown clinical specimens in three independent clinical sites. The Kappa values between *ProDx*™ HSV-1/2 DNA qPCR Detection Kit and a validated reference kit are 0.992 and 0.903 respectively, showing excellent agreement for the test results between the two kits.

Clinical data from three sites:

Clinical data from three sites:

	Validated Reference Kit for HSV-1 DNA			
***ProDx*™ HSV-1/2 DNA qPCR Detection Kit**		**Positive**	**Negative**	**Total**
	Positive	74	1	75
	Negative	0	590	590
	Total	74	591	665
	Validated Reference Kit for HSV-2 DNA			
***ProDx*™ HSV-1/2 DNA qPCR Detection Kit**		**Positive**	**Negative**	**Total**
	Positive	186	21	207
	Negative	6	452	458
	Total	192	473	665

	HSV-1	**HSV-2**
Clinical detection sensitivity	**100%**	**96.9%**
Clinical detection specificity	**99.8%**	**95.6%**

13. REFERENCES

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