OCTOBIO

OCTO Health RT-PCR NON-INVASIVE GENDER DETERMINATION KIT

Catalog #	Description
RT-NIGD-25	Real-Time PCR Non-Invasive Gender Determination Kit, 25 reactions
RT-NIGD-50	Real-Time PCR Non-Invasive Gender Determination Kit, 50 reactions

IVD

CE

Introduction

The OCTO HEALTH RT-NIGD kit is intended for non-invasive fetal sex determination by detection of Y-chromosome cell-free fetal DNA (cffDNA) in maternal plasma. The assay is performed on cell-free DNA extracted from maternal plasma and provides a qualitative result based on real-time PCR amplification:

- FAM channel: Y-chromosome target (presence indicates Y-chromosome DNA detected)
- HEX channel: Internal Control (IC) human gene to confirm adequate nucleic acid presence and PCR performance

Results must be interpreted together with control results and internal control performance criteria.

Principle of the Test

The assay is based on **real-time PCR amplification** using target-specific primers and fluorescent hydrolysis probes. During amplification, fluorescence increases proportionally to product generation, and the instrument calculates a **Ct (cycle threshold)**.

This kit includes an **Internal Control (HEX)** amplifying a human gene from the extracted maternal plasma cfDNA (endogenous control). The Internal Control is used to verify:

- Sufficient nucleic acid input/extraction success, and
- Absence of significant PCR inhibition.

Kit Contents

	CONTENT	25 RXN	50 RXN
1	Gender Determination Mix	375 µl	750 µl
2	Positive Control (PC)	50 µl	100 µl
3	Negative Control (NC)	50 µl	100 µl

Required Equipment and Materials:

- Real-time PCR instrument capable of detecting **FAM** and **HEX** channels
- PCR tubes/plates and optical seals compatible with the instrument
- Calibrated micropipettes and aerosol-resistant filter tips
- Mini-centrifuge and vortex mixer
- Clean bench / PCR cabinet (recommended)
- PPE (laboratory coat, gloves, eye protection)
- Cell-free DNA extraction kit (recommended): Qiagen circulating/cell-free DNA extraction kit suitable for plasma cfDNA
  (Follow the extraction kit manufacturer's instructions, including recommended elution volume.)

* *Please contact for other compatible devices*

Storage Conditions

- All components of the kit should be stored at -20°C until the expiration date.
- Kit components should undergo freeze-thaw cycles a maximum of 5 times. If more than 5 freeze-thaw cycles are anticipated, it is necessary to divide the kit components into smaller volumes for storage.
- Negative Control included in the kit should be stored at 2-8 °C after the initial thawing.

Experimental Preparation

Preparation of Materials:

- Materials stored at -20°C should be thawed on ice or cooling rack.
- Once thawed, liquid materials should be mixed well by pipetting.

Preparation of Reactions:

1. Preparation of 1 tube is required for each sample. Reaction volumes are specified in the table. The same tubes should be prepared for the NTC.

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Content	Amount (µl)
PCR Master Mix	15
DNA Isolate / NTC	5
TOTAL	20

Real – Time PCR Protocol

Cycle	Temperature (°C)	Time (Seconds)	Fluorescent Dye
1	95 °C	300 s	
40	95 °C	1 s	
	60 °C	1 s	FAM, HEX

Note: Very short step times require an instrument capable of stable temperature control and rapid fluorescence acquisition. Laboratories should verify protocol performance on their specific platform during validation.

Data Analysis – Interpretation of Results

A run is valid only if all controls meet the criteria below

- Positive Control (PC) must be positive
  - FAM: Detected (Ct < 35)
  - HEX: Detected (Ct < 35)
  - If PC fails in either channel, invalidate the run and repeat after checking reagents and instrument settings.
- Negative Control (NC)
  - FAM: Not Detected
  - HEX: Detected (Ct < 35)
  - If NC show FAM amplification, suspect contamination and invalidate the run.
- No Template Control (NTC)
  - FAM: Not Detected
  - HEX: Not Detected
  - Any amplification in the NTC indicates contamination. Invalidate the run and repeat after decontamination and reagent review.
- Sample
  - HEX: Detected (Ct ≤ 35)
    - Sample is valid for interpretation
  - HEX: Detected (Ct > 35)
    - Invalid Sample (Insufficient DNA and/or inhibition)
    - Repeat PCR once, if the issue persists, repeat extraction and/or request new specimen.

TUBE 1	Emission Ch.	Ct	Interpretation
Internal Control	HEX	≤ 35	Valid Sample
		> 35 or Not Detected	Invalid Sample (Insufficient cfDNA or Inhibition)
Y-Chromosome	FAM	≤ 35	Y-Chromosome Detected - Male
		Not Detected	Y-Chromosome Not Detetected - Female

Borderline / Inconclusive Situations

The following cases should be handled as **repeat-required** to minimize false calls:

- FAM detected very late (e.g., **Ct 38–40**) especially if tested in a single replicate
- Duplicate tests are discordant (one replicate FAM positive, the other negative)
- Control Ct values drift outside laboratory historical ranges

Recommended action:

1. repeat PCR from the same extract;
2. if still borderline, repeat extraction from reserved plasma (if available) or request a new specimen.

Limitations

- **Low fetal fraction**, especially early in pregnancy, may lead to **false "female"** or **inconclusive** results. Laboratories should define minimum gestational age and repeat strategy based on their validation.
- Pre-analytical variables (blood tube type, time to plasma separation, centrifugation conditions, storage) can significantly affect cfDNA quality.
- Multiple gestation, vanishing twin, recent maternal transfusion, transplantation, or other atypical clinical conditions may affect interpretation.
- PCR inhibition or low cfDNA yield may result in **HEX IC failure** (invalid results).

Troubleshooting

1. PC Fails (FAM and/or HEX not Detected)
   - *Possible causes:* Incorrect cycling, reagent degradation, instrument settings.
     - *Solutions:* Verify protocol and channels, confirm -20°C storage, repeat run with fresh aliquots.
2. NC shows FAM signal.
   - *Possible causes:* Contamination
   - *Solutions:* Invalidate the run, decontaminate workspace, replace top/reagents, repeat with new aliquots.
3. NTC shows amplification
   - *Possible causes:* Contamination
   - *Solutions:* Decontaminate, prepare fresh master mix, ensure workflow separation.
4. HEX Ct > 35 in many samples
   - *Possible causes:* Low cfDNA yield, extraction issue, inhibitors
   - *Solutions:* Review plasma prep, verify extraction, repeat extraction, check for inhibitors.
5. Late/Weak FAM signal (Ct > 38)
   - *Possible causes:* Low fetal fraction, borderline positivity
   - *Solutions:* Repeat PCR and consider duplicate testing; repeat extraction if needed

Warnings and Precautions

This test is for in vitro diagnostic purposes only and should be used strictly in accordance with the instructions provided by trained personnel. Clinical samples should be considered as materials potentially carrying an infection risk and should be prepared in a laminar flow cabinet.

• Do not use expired kit. • Avoid repeated freezing and thawing of reagents as it may reduce the sensitivity of the test. • Ensure that reagents are thawed before use, then gently mix the tubes and centrifuge briefly. • Prepare the reaction mixture quickly on ice or a cold block. • Establish two separate work areas, one for isolation and the other for PCR. • Avoid transferring pipettes, tubes, and other working materials between the work areas. • Always use filtered sterile pipette tips. • Use separate lab coats and gloves for each work area.

Symbols Used

The following symbols used on labels and packaging of this product conform to the harmonized standard **TS EN ISO 15223-1**



:Number of Reactions



: Expiration Date



: Lot Number



: Reference Number



: Temperature Limit



: Refer to the User Manual



: Manufacturer



: Keep Away From Direct Sunlight

Octo Biotechnology R&D Inc.
REŞİTPAŞA Mah. KATAR Cad. ARI 4 Binası No: 2 /50/6 SARIYER / İSTANBUL ***T:*** 0090 212 286 1368 | +90 555 331 7254
info@octobiotechnology.com www.octobiotechnology.com

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