

WizDx™ CrystalMix Bovine ID

INTENDED USE

WizDx™ CrystalMix Bovine ID kit provides a range of testing options for the real-time detection of Bovine species to ensure compliance with regulations on the labeling of foods and composition of animal feeds. WizDx™ CrystalMix Bovine ID kit is for research use only.

PRINCIPLES OF THE TEST

WizDx™ CrystalMix Bovine ID combines all reagents necessary for successful real-time PCR in a convenient individual aliquot and lyophilized in an 8-strip qPCR tube. Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target-specific probes for the Bovine. The UDG system is applied to prevent carry-over contamination, and the Uracil DNA Glycosylase treatment and qPCR (Real-time PCR) are continuously performed in one tube, so it is highly convenient for the user and can prevent contamination between samples or amplicons. The probes are Dual-labeled with a fluorescent reporter and quencher dyes. For each labeled probe, probes specific for the Bovine labeled with the fluorophore FAM. The probe is specific for the Internal Positive Control (IPC) is labeled with the fluorophore Cy5 to monitor for PCR inhibition and to validate the quality of the sample and detection result.

KIT STORAGE AND STABILITY

- Store at 4 - 25°C, **Do not freeze Bovine ID CrystalMix.**
- **Do not use it once the cone-shape mix shrinks as a dot form. It was damaged by rehydration.**
- Expires 12 months from the date of manufacture.

KIT CONTENTS

Component	Amount	Cap Color
Bovine ID CrystalMix	96 tubes	
Bovine ID Positive control *	1 vial	●
Deionized sterile Water *	1 vial	○

* Before using the positive control, add 200µl of Deionized sterile Water and dissolve sufficiently.

* Positive Control and Deionized sterile Water should be stored and shipped at room temperature. Once opened, please store them at -20°C.

REAGENT AND EQUIPMENT TO BE SUPPLIED BY THE USER

- Real-Time PCR System
- Micropipette & sterile pipette tips
- Vortex mixer & microcentrifuge
- Protective ware & disposable gloves

QUALITY CONTROL

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizDx™ CrystalMix Bovine ID kit is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTION

- For research use only.
- For single use only. Do not reuse.
- Do not drink RNase-free water.
- Carefully read this instruction before use.
- Do not use any reagents after the expiration date.
- Always wear personal protective equipment (gloves, mask, etc.) when handling biohazardous agents in compliance with relevant regulations.
- Always use sterile, filtered pipette tips.
- Take care of the handling of specimens to minimize the risk of infection.
- Dispose of waste in compliance with relevant regulations after the test.

PROTOCOL

- Please read through the entire protocol before starting.
- Use the required number of tubes and immediately put the remaining tubes in the pouch and seal with the zipper.

[Step 1] DNA Preparation

Please carry out the DNA isolation according to the manufacturer's instructions. The following standard extraction kit is recommended.

- WizMag™ Cell/Tissue DNA (REF. W7040, Wizbiosolutions Inc.)
- WizPrep™ gDNA Mini Kit (Cell/Tissue) (REF. W71060, Wizbiosolutions Inc.)

[Step 2] Prepare PCR Reaction

1) Prepare the PCR reaction as the following table.

Component	Sample	PC *	NTC *
Bovine ID CrystalMix	1 tube	1 tube	1 tube
Sample (DNA)	20 µL	-	-
Bovine ID Positive Control	-	20 µL	-
Deionized sterile Water	-	-	20 µL
Total	20 µL	20 µL	20 µL

* PC: Positive control, NTC: Non-template control

To avoid contamination, close the cap immediately after placing the sample in the tube.

2) Vortex for 3 - 5 sec. and briefly spin down.

3) Insert the CrystalMix tube into the Real-Time PCR System.

[Step 3] Run Real-time PCR

1) Prepare the Real-time PCR program set-up as the following table.

Step	Temp.	Time	Cycle
UDG Treatment (Carryover prevention)	50 °C	5 min.	1
Pre-Denaturation	95 °C	3 min.	1
Denaturation	95 °C	10 sec.	40
Annealing (Probe detection)	55 °C	30 sec.	

2) Set up the threshold and baseline of the fluorescence probes as follows:

PCR System	Fluorescence	Threshold	Baseline	
			Begin	End
CFX96™	FAM	100	Auto	
	Cy5	50		
QuantStudio5	FAM	Auto		
	Cy5			
CLEO™ Q16	FAM	Auto		
	Cy5			

[Step 4] Data Analysis and Interpretation

The following results are possible:

- 1) A signal is detected in channel FAM. The result is positive: The sample contains Bovine DNA. In this case, the detection of a signal in channel Cy5 (Internal Positive Control ;IPC) is dispensable, as high initial concentrations of Bovine DNA. can lead to a reduced or absent fluorescence signal of the IPC (competition).
- 2) In channel FAM no signal is detected, At the same time, a Cy5 signal from the IPC appears. The sample does not contain any Bovine DNA. It can be considered negative. In the case of a negative Bovine PCR the detected signal of the IPC rules out the possibility of PCR inhibition.

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3) Neither in channel FAM nor in channel Cy5 is a signal detected. A diagnostic statement can not be made. Inhibition of the PCR reaction.

Target	FAM	Cy5	Interpretation
Positive Control	+	+	Positive/Valid
Negative Control	-	+	Negative/Valid
Sample	+	+	Positive/Valid
Sample	+	-	Positive/Valid
Sample	- (> Ct*)	- (> Ct*)	Invalid
Sample	-	-	Invalid

* For Ct values, see Important product information bulletin

- If the result is positive in both Cy5 and FAM channels, the result is valid, Bovine DNA is detected.
- If the result is negative in the FAM channel and the result in the Cy5 channel is positive, the result is valid, Bovine DNA is not detected.
- If the result is negative or > Ct (for different thermocyclers) in both Cy5 and FAM channels, the result is invalid. It is necessary to repeat amplification. If the result is the same, repeat DNA extraction. If the result is the same again, it is considered to be invalid. In this case, it is recommended to repeat material sampling.
- If the result is > Ct in the FAM channel and the result in the Cy5 channel is positive, the result is invalid. It is necessary to repeat amplification. If the result is the same, repeat DNA extraction. If the result is the same again, it is considered to be equivocal. In this case, it is recommended to repeat material sampling.

TROUBLE SHOOTING GUIDE

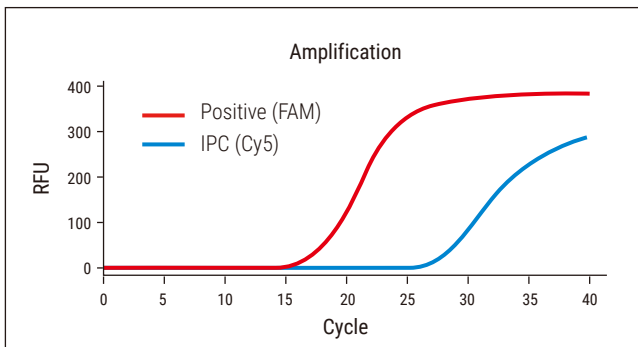
- In the case of difficult to interpret results due to non-specific amplification.
 - Reduce amount of template by 1/10 dilution and reacts again.
- Preparation of PCR reaction at room temperature may cause the non-specific amplification.

SYMBOL GLOSSARY

Symbol	Meaning	Symbol	Meaning
REF	Catalogue number		Manufacturer
LOT	Batch code	RUO	Research use only
	Temperature limit		Consult instructions for use
	Use-by date	Σ	Contents sufficient for <n> tests
	Caution		Keep away from sunlight
	Do not reuse		Keep dry

ORDERING INFORMATION

Product	Cat No.	Package
WizDx™ CrystalMix Bovine ID	DX1211	96 Test
WizPrep™ gDNA Mini Kit (Cell/Tissue)	W71060	100 prep
WizMag™ Cell/Tissue DNA	W7040	64 prep
CLEO™ Q16 Real-time PCR system	CL0016	1 system
CLEO™ AP16 Nucleic Acid Extractor	CL2016	1 system



Technical Support

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