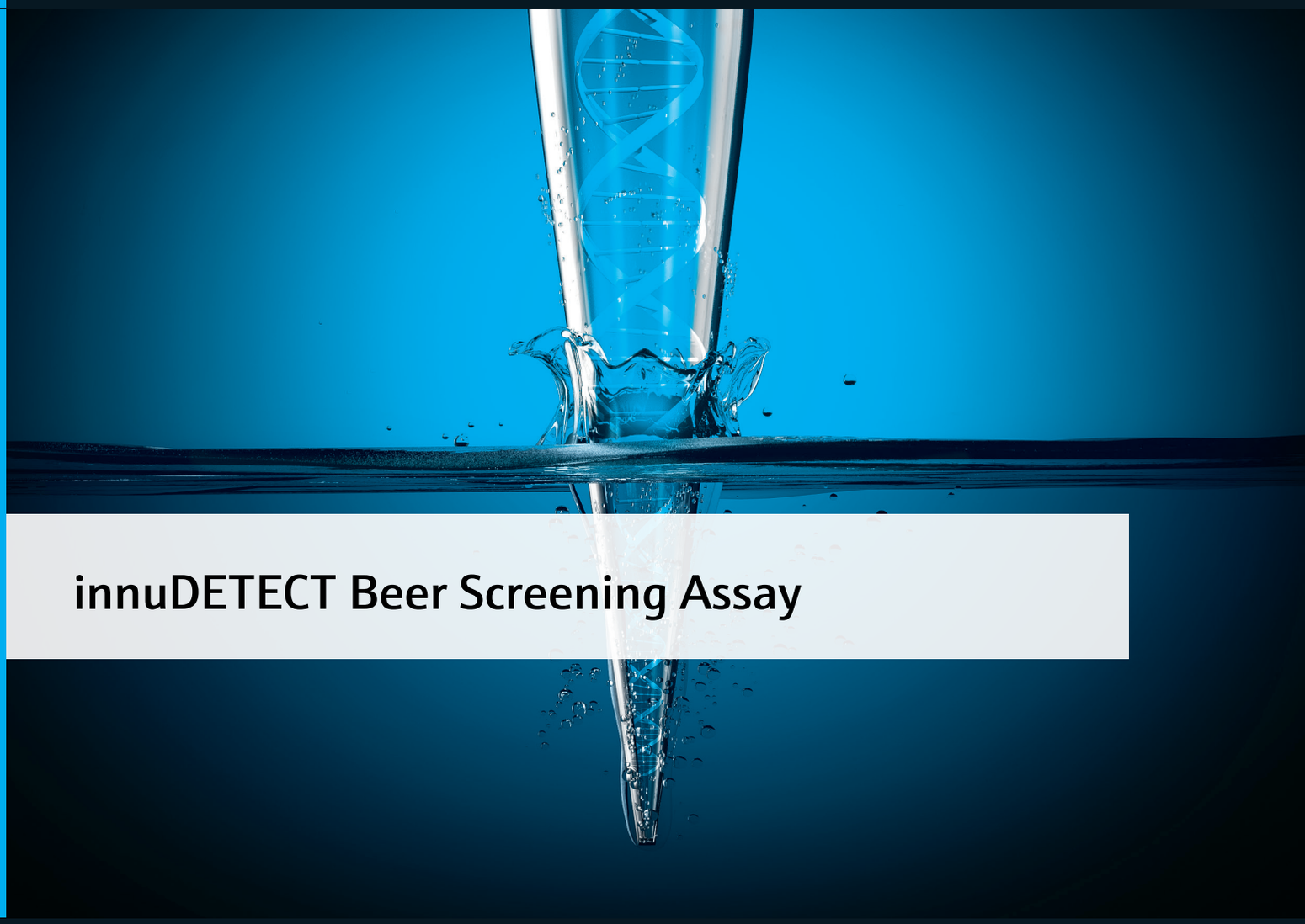
Instructions for Use
Life Science Kits & Assays



innuDETECT Beer Screening Assay

iST Innuscreen GmbH

1 Product specifications

Starting material	Isolated total DNA from beer samples
Time of detection	~ 60 minutes
qPCR detection channels	FAM (Target) and HEX (IC)
Sensitivity	Up to 10 DNA copies/PCR

Detection of DNA isolated from beer samples or shaking culture using innuPREP TCT Beer Extraction Kit from IST Innuscreen GmbH. (see Related Products). Please make sure that the common quality requirements for DNA samples are achieved.

2 Intended use

The innuDETECT Beer Screening Assay is a molecular diagnostic test system for detection of beer spoilage bacteria and hop resistance genes based on TaqMan® principle. The Assay can be used with all commonly RealTime cyclers detecting FAM and HEX dyes. The Assay PCR MasterMix does not include any reference dyes.

The assay includes as Positive Control (PC) the DNA from hop resistance gene positive *Pediococcus damnosus* (DSM 20331),

The assay includes an Internal Control (IC) that can be used as amplification control if added to the PCR reaction. If added to the Lysis Buffer the IC can also be utilized to check the DNA-extraction method used.

The Assay includes two Primer/Probe Mixes:

1. Primer/Probe Mix Bacteria IC

Beer spoilage bacteria detected				
Lactobacillus		Pediococcus	Pectinatus	Megasphaera
L. brevis	*L. acetotolerans*	*Ped. acidilactici*	*Pect. cerevisiiphilus*	*M. cerevisiae*
L. plantarum	*L. paraplantarum*	*Ped. pentosaceus*	*Pect. frisingensis*	*M. stantonii*
L. backii	*L. rhamnosus*	*Ped. parvulus*	*Pect. portalensis*	*M. elsdenii*
L. paracasei	*L. pentosus*	*Ped. claussenii*	*Pec. haikarae*	*M. paucivorans*
L. buchneri	*L. coryniformis*	*Ped. stilesii*		
L. casei	*L. sakei*	*Ped. inopinatus*		
L. reuteri	*L. parabuchneri*	*Ped. damnosus*		
L. lindneri	*L. collinoides*			
L. perolens	*L. paracollinoides*			
L. rossiae	*L. harbinensis*			

2. Primer/Probe Mix HoR IC

Hop resistance genes detected
Hor A
Hor C
Hit A

3 Product and order number

Name	Amount	Order-no.
innuDETECT Beer Screening Assay	24 rxn	845-IDF-0150024

4 Storage conditions

The Assay is delivered at ambient temperature.
Store the innuDETECT Beer Screening Assay at -22 °C to -18 °C, except the innuDRY qPCR MasterMix Probe that should be stored before dissolving at 4 °C to 8 °C.
When stored as recommended, the kit is stable until the expiration date printed on the label on the kit box.

5 Delivered components

Components		Σ 24
Primer/Probe Mix Bacteria IC		75 µl
Primer/Probe Mix HoR IC		75 µl
innuDRY qPCR MasterMix Probe		2
Resuspension Buffer Probe		2x300 µl
Positive Control		30 µl
Internal Control		1
PCR-grade H_2O		2 ml

6 Safety precautions

The assay shall only be handled by educated personal in a laboratory environment. The compliance with the specified procedure is absolutely mandatory when performing this assay.

Reagents should be stored in their original containers at the indicated temperatures. Do not replace individual components with those from different batches or test assays. Note the indicated expiration dates.

Do not eat, drink or smoke while performing the assay.

Wear protective clothing and safety gloves.

All samples and test materials should be handled and disposed of as infectious material, in accordance with regulatory requirements.

Reagent containers that have not come in contact with potentially infectious material may be disposed of along with ordinary laboratory waste.

Store the reagents used for performing PCR separately from DNA templates and amplification products.

7 Reagent preparation

7.1 Internal Control

Dissolve the lyophilized Internal Control (IC) by adding 1.25 ml of PCR-grade H_2O and mix thoroughly.

To use the IC as an extraction control, add to the Lysis Buffer/Sample Mix amount of IC which is 1/10 from final elution volume (see according DNA isolation instruction manual). Use the co-amplification of spiked IC to observe the relative loss of DNA during the extraction procedure.

To use the IC as an amplification control, **dilute** the dissolved IC **1:100** and add 1 µl of diluted IC to each PCR reaction.

Alternatively, the 1:100 diluted IC can be added to the qPCR reaction mix in an amount of 1 µl/reaction. NOTE: in this case the No Template Control (NTC) must also be positive for IC.

7.2 2x MasterMix

The 2x MasterMix must be prepared before starting the PCR setup and can be stored at -22 °C to -18 °C.

Add 250 µl for 24 rxn assay or 1 ml for 96 rxn assay of Resuspension Buffer Probe to the innuDRY qPCR MasterMix Probe tube. Vortex carefully and centrifuge the tube to collect the liquid on the bottom.

8 Real-Time PCR (qPCR)

8.1 Setup of the experiment

It is recommended that one first determine whether the beer sample contains the bacteria that can potentially cause beer spoilage. If the result is positive, the hop resistance genes can be detected. However, both PCR reactions can be performed simultaneously on one plate.

8.2 Preparation of reaction batches

Determine the total number of required qPCR reactions considering also at least one NTC.

Reagent	Volume (1 rxn)
2x MasterMix	10 µl
Primer/Probe Mix Bacteria IC or Primer/Probe Mix HoR IC	3 µl
IC (1:100 diluted if used as amplification IC)	1 µl
Sample (PCR-grade H_2O for NTC) or PC (if needed)	≤ 2 µg DNA, max 5 µl 1 µl
PCR-grade H_2O	Fill up to 20 µl

The composition of the qPCR reaction mix for one sample is shown in the table below. Prepare the qPCR reaction mix for the number of samples needed (including NTC).

Add qPCR reaction mix to PCR stripe or plate. In a second step add samples to the qPCR reaction mix in order to avoid the cross contamination.

Seal the PCR stripe or plate with an appropriate sealing film (PP) and/or cap; place tubes in the Real-Time PCR Cycler and close the lid.

8.3 Real-Time PCR conditions

For basic information regarding the setup and programming of the different Real-Time PCR Cycler, please refer to the manual of the respective instrument.

Program the Real-Time PCR Cycler as indicated in the table below and start the program.

Step	Cycles	Profile	Temperature	Retention time
1	1	Initial denaturation	95 °C	120 s
2	40	Denaturation	95 °C	10 s
		Annealing / Elongation*	58 °C	45 s

* Data acquisition: Fluorescence Detection (FAM; HEX)

9 Interpretation of results

Please refer to the following table to identify the signal pattern that matches to the obtained signals. It is strongly recommended to run at least one No Template Control (NTC) for each experiment.

The Ct value of IC can vary (or even disappear) in dependence of DNA quality and intensity of FAM signal.

FAM	HEX	Sample	Valid	Recommended interpretation
+	**(+)**	NTC	no	Contamination of PCR chemicals with target or (and) IC DNA
-	**+**	NTC	yes	No contamination
+	**(+)**	PC	yes	PCR run successful
+ or-	**+**	Unknown Sample	yes	Positive or negative for the target
-	**-**	Unknown Sample	no	PCR reaction and/or DNA isolation failed
++	**-**	Unknown Sample	yes	The sample is strong positive for the target

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