



foodproof® Animal Detection 2 LyoKit

Revision A, November 2025

PCR kit for the qualitative detection of chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*) and poultry (birds) in one multiplex assay using real-time PCR instruments.

Product No. KIT230032

Product No. KIT230033

Kit for 96 reactions (lyophilized) for a maximum of 94 samples

Store the kit at 2 to 8 °C

For food testing purposes.

FOR IN VITRO USE ONLY



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1. Product Overview

1.1 Product Information

The foodproof® Animal Detection 2 LyoKit was developed to reliably detect DNA of chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*) and poultry DNA in raw material, food- and feed products with real-time PCR instruments. In case of false labeling of food products the foodproof® Animal Detection 2 LyoKit enables a very sensitive analysis by using amplification of multi copy targets with chicken, turkey and bird (whole class of Aves, including goose (*Anser*), duck (*Anas*), ostrich (*Struthio*), chicken (*Gallus*), turkey (*Meleagris*), guinea fowl (*Numida*) and other birds) specific primers and probes. The detection limit is up to 1 ppm chicken, turkey and poultry in meat products. Because of the high sensitivity of the test, the kit is suitable for analyzing highly processed products. Control Template and Internal Control, as well as Negative Control, monitor the PCR run validity. PCR results, including the DNA extraction process, are obtained in less than two hours.

If quantification of meat samples is desired, it is possible to refer to our reference material Animal RM 2: DNA of raw meat mixtures from 1%, 0.1%, 0.01% chicken- and turkey- in beef meat.

Note: For quantification details, please contact us: www.hygiena.com

1.2 Product Characteristics

Specificity	The primers and hydrolysis probes (5' nuclease probes) provided in the lyophilized mix are sequence-specific for chicken, turkey and poultry/bird DNA and the Internal Control, respectively. The specificity of the assay was proven by more than 50 different animals, microorganisms and plant species.
Sensitivity	The limit of detection: 1 genome equivalent of <i>Gallus gallus</i> , <i>Meleagris gallopavo</i> and <i>Anser anser</i> for poultry, as well as 1 ppm/0.0001% chicken and turkey in beef meat.
Matrix	Kit was validated with meat products (e.g., instant food, feed and egg products).
Robustness	Reproducibility of Ct-values was successfully tested with different Real-Time PCR instruments, including LightCycler® 480 II, LightCycler® 96 (Roche); AriaMx (Agilent Technologies); Mx3005P® (Agilent Technologies); Applied Biosystems® 7500 FAST; CFX96™ Real-Time PCR Cycler (Bio-Rad).

Note: More detailed information is listed in the Validation Data Report of the foodproof Animal Detection 2 LyoKit. Please contact our Technical Support (hygiena.com/support).

1.3 Number of Tests

The kit is designed for 96 reactions with a final reaction volume of 25 µL each. Up to 94 samples (single sample preparation) plus one positive (purple cap) and one negative control reaction (colorless cap) can be analyzed per run.

1.4 Storage and Stability

- Store the kit at 2 °C to 8 °C through the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following kit contents table:



1.5 Kit contents

Component	Label	Contents / Function / Storage
foodproof Animal Detection 2 LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat • KIT230032 with white low-profile tubes* • KIT230033 with clear regular profile tubes*	• 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing • primer and hydrolysis probes specific for chicken (<i>Gallus gallus</i>), turkey (<i>Meleagris gallopavo</i>) and poultry DNA (whole class of Aves) and the Internal Control (IC) • Internal Control Plasmid • Taq DNA Polymerase • Uracil-DNA N-Glycosylase (UNG, heat labile) for prevention of carry-over contamination • For amplification and detection of chicken (<i>Gallus gallus</i>), turkey (<i>Meleagris gallopavo</i>) and poultry DNA (whole class of Aves), for internal amplification control in case of inhibition • Store at 2 °C to 8 °C in the aluminum bag (sealed) • Protect from light and moisture!
Control Template	Vial 2 (purple cap)	• 1 x 250 µL • Contains a stabilized solution of chicken (<i>Gallus gallus</i>), turkey (<i>Meleagris gallopavo</i>) and poultry DNA • For use as a PCR run positive control. • Store at 2 to 8 °C.
H ₂ O PCR-grade	Vial 3 (colorless cap)	• 2 x 1 mL • Nuclease-free, PCR-grade H₂O. • For use as a PCR run negative control.
Cap strips	Plastic bag containing 8-cap strips	• 12 x 8-cap strip • For use in real-time PCR after addition of samples.

*Tube profile and instrument **compatibility** chart is available on hygiena.com/documents

1.6 Additional Equipment and Reagents Required

- Real-time PCR instrument with a FAM, HEX, ROX, and Cy5 detection channel, capable of performing a melting curve analysis. In cases where the low or regular profile strip tubes do not fit into the instrument, the samples have to be transferred after resuspension of the lyophilized PCR mix to appropriate PCR vessels.
- Sample Preparation Kit
 - foodproof StarPrep® Five Kit (Product No.: KIT230191), or
 - foodproof Magnetic Preparation Kit III for animal identification (Product No.: KIT230182), or
 - foodproof Sample Preparation Kit III for animal identification (Product No.: KIT230174)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes



1.7 Applicability Statement

The foodproof Animal Detection 2 LyoKit is intended for the rapid detection of chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*) and poultry DNA (whole class of Aves) in processed food or feed samples.

The foodproof Animal Detection 2 LyoKit has been developed for real-time PCR instruments with a FAM, HEX, ROX, and Cy5 detection channel. The performance of the kit was tested with the following real-time PCR instruments: LightCycler 480 II, LightCycler 96 (Roche); AriaMx, Agilent Mx3005P® (Agilent Technologies); Applied Biosystems 7500 FAST (Thermo Fisher Scientific); CFX96™ real-time PCR Cycler (Bio-Rad).

2. Procedure

2.1 Before You Begin

2.1.1 Precautions

The kit provides all reagents required for the PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease, carry-over, or cross-contamination:

- Keep the kit components separate from other reagents in the laboratory.
- Use nuclease-free labware (e.g., pipettes, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-preventive pipette tips.
- To avoid carry-over contamination, transfer the required solutions for one experiment into a fresh tube, rather than directly pipetting from stock solutions.
- Physically separate the workplaces for DNA preparation, PCR setup, and PCR to minimize the risk of carry-over contamination. Use a PCR hood for all pipetting steps.

Keep the foodproof Animal Detection 2 LyoKit PCR Mix away from light and moisture.

2.1.2 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration and absence of inhibitors. For the preparation of DNA from raw material of animal origin food, feed, pharmaceutical, gelatin and milk products, refer to the corresponding product package inserts of a suitable sample preparation kit (see “Additional Equipment and Reagents Required”).

2.1.3 DNA Extraction

Hygiena Diagnostics provides sample preparation kits suitable for all kinds of raw materials from animal origin food, feed or pharmaceutical, gelatin and milk products.

- foodproof StarPrep Five Kit (Product No.: KIT230191)
- foodproof Magnetic Preparation Kit III (Product No.: KIT230182)
- foodproof Sample Preparation Kit III (Product No.: KIT230174)

(see “Additional Equipment and Reagents Required”).

For more product information, please refer to www.hygiena.com



2.1.4 Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [foodproof Animal Detection 2 LyoKit Control Template (vial 2, purple cap)] or with a positive sample preparation control.

2.1.5 Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H₂O, PCR-grade water (vial 3, colorless cap). Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.

2.2 Program

2.2.1 Program Setup

The following procedure is optimized for a real-time PCR instrument with a FAM, HEX, ROX, and Cy5 detection channel.

Program the PCR instrument before preparing the samples. Use the following real-time PCR protocol for the foodproof Animal Detection 2 LyoKit (for details on how to program the experimental protocol, see the operator's manual of your real-time PCR cycler):

Real-time PCR Time-Temperature Protocol	
<u>Pre-incubation</u>	1 cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 5 minutes
<u>Amplification</u>	35 cycles
Step 1:	95 °C for 5 seconds
Step 2*:	60 °C for 60 seconds
* Fluorescence detection in step 2	

Notes:

- For some real-time PCR instruments, the type of probe quencher as well as the usage of a passive reference dye must be specified. The foodproof Animal Detection 2 LyoKit contains probes with a nonfluorescent ("dark") quencher as the quencher and no passive reference dye.
- A Color Compensation Set (Color Compensation Set 3; Product No.: KIT230005) is necessary and will be supplied by Hygiena Diagnostics for users of the LightCycler 480 Systems I and II. Please contact Hygiena Diagnostics for further information.



2.2.2 Preparation of the PCR Mix

Proceed as described below to prepare a 25 µL standard reaction. Always wear gloves when handling PCR strips or caps. The lyophilizate is only stable in the provided aluminum bag with the silica gel pad.

1. Take the needed number of PCR tube strips out of the aluminum bag. Close the bag afterwards.
2. Place the PCR tube strips containing the lyophilized reagents in a suitable PCR tube rack. Check that the reagent pellets are at the bottom of the tubes. If not, briefly centrifuge or flick the pellets to the bottom before proceeding.
3. Decap the tube strips cautiously and discard the clear cap strips.
Note: Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, open strips only shortly before filling.
4. Pipette a total volume of 25 µL of sample into each PCR vessel and resuspend the pellet by cautiously pipetting up and down:
 - For samples prepared with the foodproof StarPrep Five Kit: Pipette 22 µL PCR-grade H₂O into the PCR vessel, then add 3 µL sample DNA.
 - For samples prepared with the foodproof Sample Preparation Kit III or foodproof Magnetic Preparation Kit III: Pipette 25 µL of sample DNA into the PCR vessel.**Note:** If inhibition of PCR occurs, use a smaller sample volume and add to 25 µL with H₂O.
5. For the negative control, add 25 µL H₂O, PCR-grade (vial 3, colorless cap).
6. For the positive control, add 25 µL Control Template (vial 2, purple cap).
7. Seal the vessels accurately with the colorless cap strips.
Note: Alternatively, resuspend the pellet after sealing by mixing thoroughly. To reduce the risk of cross-contamination, it is recommended to prepare only one PCR tube strip at once.
8. Briefly spin the PCR tube strips in a suitable centrifuge.
9. Cycle the samples as described above.
Note: For using the LightCycler 480 instrument, a special adapter is necessary. For some PCR instruments, the PCR strips should be placed in a balanced order into the cycler block. For example, two strips can be placed in columns 1 and 12.

2.3 Data Interpretation

2.3.1 Result Interpretation of the Amplification Curves

- A positive amplification signal in the FAM detection channel indicates the presence of chicken (*Gallus gallus*) DNA in the tested sample.
- A positive amplification signal in the HEX detection channel indicates the presence of turkey (*Meleagris gallopavo*) DNA in the tested sample.
- A positive amplification signal in ROX detection channel indicates the presence of bird/poultry (Aves) DNA in the tested sample.
- Positive amplification signals in FAM, HEX, and ROX detection channels indicate the presence of chicken, turkey DNA and/or DNA from other birds in the tested sample.

To exclude PCR inhibition, the amplification in the Cy5 detection channel must be checked, if there is a negative result in all channels. If all results are negative, the total result is invalid.



Channel FAM	Channel HEX	Channel ROX	Channel Cy5	Result Interpretation
Positive	Negative	Positive	Positive or Negative	Positive for <i>Gallus gallus</i>
Negative	Positive	Positive	Positive or Negative	Positive for <i>Meleagris gallopavo</i>
Negative	Negative	Positive	Positive or Negative	Positive for Aves, negative for <i>Gallus gallus</i> , <i>Meleagris gallopavo</i>
Positive	Positive	Positive	Positive or Negative	Positive for <i>Gallus gallus</i> , <i>Meleagris gallopavo</i> , Aves
Negative	Negative	Negative	Positive	Negative for <i>Gallus gallus</i> , <i>Meleagris gallopavo</i> , Aves
Negative	Negative	Negative	Negative	Invalid

Note: A prerequisite for a clear assignment of the chicken, turkey and Aves/poultry DNA is an adequate calibration of the PCR cyclers for the channels FAM, HEX, ROX and Cy5. Please refer to the operating manual of your real-time PCR Cycler for further information.



3. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase, even with positive controls.	Incorrect detection channel has been chosen.	Set channel settings of FAM, VIC/HEX, ROX and Cy5.
	Pipetting errors.	- Check for correct reaction setup. Repeat the PCR run. - Always run a positive control along with your samples.
	No data acquisition programmed.	Check the cycle program.
No signal increase in channel Cy5.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	Dilute samples or pipette a lower amount of sample DNA (e.g., 20 µL PCR-grade H₂O and 5 µL sample DNA instead of 25 µL sample DNA).
	Large amounts of chicken, turkey and Aves/poultry DNA	If a positive signal in FAM, HEX or ROX is detected, the PCR run is valid.
Fluorescence intensity is too low.	Inappropriate storage of kit components.	Store the lyophilized PCR-mix foodproof Animal Detection 2 LyoKit at 2 to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
Strong decrease of fluorescence baseline.	Resuspension of lyophilized PCR-mix not complete.	Always resuspend lyophilized PCR-mix thoroughly.
Negative control samples are positive.	Carry-over contamination.	- Exchange all critical solutions. - Repeat the complete experiment with fresh aliquots of all reagents. - Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination. - Add positive control after all sample and negative control reaction tubes have been sealed.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR strips. Resuspension of the PCR-mix only in the upper part of the reaction tube.	Always centrifuge PCR strips.
	Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	Always wear gloves when handling the vessels and seal.
Pellets are hard to dissolve.	The lyophilized PCR-mix started to rehydrate.	- Store the lyophilized PCR-mix tightly sealed in the aluminum bag with silica gel pad. - Open strips just before filling.



4. Additional Information on this Product

4.1 How this Product Works

The foodproof Animal Detection 2 LyoKit provides all necessary reagents and a control template for reliable interpretations of results. To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is included. A hydrolysis probe was designed to bind specifically to the IC, allowing detection in the Cy5 channel, whereas the chicken DNA is detected in FAM, turkey DNA is detected in HEX. The amplification of Aves, including goose (*Anser*), duck (*Anas*), ostrich (*Struthio*), chicken (*Gallus*), turkey (*Meleagris*), guinea fowl (*Numida*) and other bird DNA, is analyzed in the ROX detection channel. In case of a negative result due to inhibition of the amplification by the sample DNA of interest, the amplification of the IC is suppressed as well, whereas a negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of chicken, turkey and other bird DNA in the sample. The foodproof Animal Detection 2 LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of chicken, turkey and bird/poultry DNA. Primers and probes provide specific detection of chicken, turkey and bird/poultry DNA in food and feed samples. The described performance of the kit is guaranteed for use on the real-time PCR instruments listed above.

4.2 Test Principle

1. Using sequence-specific primers of the foodproof Animal Detection 2 LyoKit in a polymerase chain reaction (PCR), the PCR instrument and the supplied reagents amplify fragments of chicken, turkey and Aves DNA specific sequences.
2. The PCR instrument detects these amplified fragments in real-time through fluorescence generated by cleavage of the hybridized probe due to the 5'-nuclease activity of the Taq DNA polymerase. The probe is labeled at the 5'-end with a reporter fluorophore and at the 3'-end with a quencher.
3. During the annealing/elongation phase of each PCR cycle, the probe hybridizes to an internal sequence of the amplicon and is cleaved by the 5'-nuclease activity of the Taq DNA polymerase. This cleavage of the probe separates the reporter dye from the quencher dye, increasing the reporter dye signal.
4. The PCR instrument measures the emitted fluorescence of the reporter dye.

4.3 Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination between PCRs. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions, and the pretreatment of all successive PCR mixtures with the heat-labile UNG. The UNG cleaves DNA at any site where a deoxyuridine residue has been incorporated. The resulting abasic sites are hydrolyzed due to the high temperatures during the initial denaturation step, and can no longer serve as PCR templates. The heat-labile UNG is inactivated during the initial denaturation step. Native DNA (e.g., the isolated animal genomic DNA) does not contain uracil and is therefore not degraded by this procedure. Since dTTP is replaced with dUTP and UNG is included in the foodproof Animal Detection 2 LyoKit, decontamination can be achieved with the provided reagents.



4.4 Quality Control

The foodproof Animal Detection 2 LyoKit is function tested using the LightCycler 480 System. More detailed information is listed in the Validation Data Report of the foodproof Animal Detection 2 LyoKit. Please contact our Technical Support (hygiena.com/support).

5. Supplementary Information

5.1 Ordering Information

Hygiena offers a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.hygiena.com.

5.2 License Notice

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,804,375, 5,538,848, 5,723,591, 5,876,930, 6,030,787, 6,258,569. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product solely in Food Testing Applications and Genetically Modified Organism (GMO) Testing Applications, including reporting results of purchaser's activities for a fee or other commercial consideration, and also for the purchaser's own internal research. No right under any other patent claim is conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

The purchase price of this product includes limited, non-transferable rights under U.S. Patent No. 7,687,247 owned by Life Technologies Corporation to use only this amount of the product to practice the claims in said patent solely for activities of the purchaser for bioburden testing, environmental testing, food testing) in accordance with the instructions for use accompanying this product. No other rights are conveyed, including no right to use this product for *in vitro* diagnostic, therapeutic, or prophylactic purposes. Further information on purchasing licenses under the above patent may be obtained by contacting the Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008. Email: outlicensing@lifetech.com.

5.3 Trademarks

foodproof® is a registered trademark of Hygiena Diagnostics GmbH. Other brand or product names are trademarks of their respective holders.

5.4 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, please contact our Technical Support staff (www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

5.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: F 602 79-1 (KIT230032) and F 602 79-2 (KIT230033).



6. Change Index

Revision A, November 2025:

First version of the product instructions.

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