Vegan Control™

qPCR kit for detecting animal traces in food

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

A growing number of consumers adopt a vegetarian or vegan lifestyle, driven by health-, animal- and environment-related concerns.

In response to the exponentially increasing demand of animal-free food products, it becomes necessary to certify the absence of ingredients of animal origin in raw or processed vegan food. This process is essential to maintain - more generally - consumer confidence in the supply chain and in the label claims.

The Vegan Control™ kit for qPCR enables the detection of animal traces in food and feed samples. Combined with a suitable sample preparation method such as our ExtractNow™ Vegan Control, the Vegan Control™ qPCR kit detects animal DNA in food and feed down to a threshold level of 0.1 % with a semi-quantitative output (see „Assay Characteristics“ for details).

Compared to protein-based approaches, qPCR-based detection assays for food analysis ensure high sensitivity (lower detection limit) and are particularly advantageous for testing of highly processed food samples.

TEST PRINCIPLE

Vegan Control™ Kit is a TaqMan®-based real-time PCR (qPCR) assay. The target sequence is a mitochondrial gene (cytochrome b), which is shared by all animal species and present as multi-copy gene. Targeting such region enables highly sensitive detection of small amounts of animal DNA (down to 10 pg/PCR reaction). Therefore, traces of mammalian, avian, and fish DNA are detected by this system. Please note that Vegan Control™ does not detect DNA from insects, reptiles, or shellfish.

The Vegan Control™ Kit is intended for the analysis of DNA extracted from food samples (e.g. by using ExtractNow™ Vegan Control kit, Cat. No. 607-1050, or user-based method). The entire test requires approximately 90 minutes, without the DNA extraction.

The kit contains all the necessary components to set up the qPCR: a lyophilized master mix including hot-start Taq polymerase, primers, probes, and dNTPs, Internal Control DNA and Positive Control DNA, Rehydration Buffer, and PCR grade Water. False negative results caused by PCR inhibition and/or DNA extraction issues will be reliably identified by means of the Internal Control DNA. The amplification of the Internal Control DNA is detected at 560 nm (HEX™ channel), whereas the animal-specific amplification is detected at 520 nm (FAM™ channel).

The Vegan Control qPCR Mix contains dUTP instead of dTTP to facilitate the degradation of amplicon carryover by use of uracil-DNA glycosylase (UNG). This approach greatly minimizes the probability of obtaining false-positive results. Please note that UNG is not included in the Vegan Control™ kit.

CONTENT

Each kit contains reagents for 25 or 100 reactions. The expiry date of the unopened package is marked on the package label. The kit components must be stored at +2 to +8 °C. After re-hydration, the reagents must be stored at ≤ -18 °C.

Component	25 reactions Cat. No. 370-2025	100 reactions Cat. No. 370-2100	Cap color
Vegan Control qPCR Mix	1 vial, lyophilized	4 vials, lyophilized	red
Positive Control DNA	1 vial, lyophilized	1 vial, lyophilized	green
Internal Control DNA	1 vial, lyophilized	4 vials, lyophilized	yellow
Rehydration Buffer	1 vial, 1.0 ml	2 vials, 1.0 ml each	blue
PCR grade Water	1 vial, 2.0 ml	1 vial, 2.0 ml	white

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com / www.minervabiolabs.us).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The Vegan Control™ kit contains necessary reagents for setting up the PCR.
Additional consumables and equipment are supplied by the user:

- qPCR device with filter sets for detecting the fluorescent dyes FAM™ and HEX™
- DNA-, RNA-, DNase- and RNase-free 0.2 ml and 1.5 ml reaction tubes
- Microcentrifuge for 1.5 ml and 0.2 ml reaction tubes
- Pipettes with corresponding filter tips (10, 100, and 1000 µl)
- Required for DNA extraction:
  DNA extraction kit, e.g. ExtractNow™ Vegan Control kit (Cat. No. 607-1050), or user-based method
  Ethanol > 96 % abs., heat block
- Optional for carry-over prevention: Uracil DNA glycosylase (UNG)

SPECIMEN

For sample preparation and DNA extraction, we recommend our ExtractNow™ Vegan Control kit (Cat. No. 607-1050). This kit uses a spin column-based isolation technology applicable to a broad range of food samples as starting material. The protocol for DNA extraction is described in detail in the „Instructions for Use“ of the DNA extraction kit. Extracted DNA may be stored at +2 – +8 °C for up to 6 days. Long-term storage must be at ≤ –18 °C. Frequent thawing and freezing of DNA extracts should be avoided to preserve the DNA integrity.

PRECAUTIONS

Food samples should be collected according to local standard methods and guidelines.
Vegan Control™ kit is for *in vitro* use only and can be used in research and industry for the detection of meat- and animal-based contaminations in food. Do not use for clinical or diagnostic applications or for testing human samples.
Vegan Control™ kit should be used by trained laboratory staff only. All samples should be handled with all due care and attention. Always wear a suitable lab coat and disposable gloves.
This kit does not contain hazardous substances. Waste is disposable according to local regulations.

ADDITIONAL NOTES

⇨ These instructions must be understood to successfully use the Vegan Control™ kit. The reagents supplied should not be mixed with reagents from different batches but used as an integral unit. The reagents of the kit must not be used beyond their shelf life.

⇨ Follow the exact protocol. Any deviations may affect the test method and results.

⇨ PCR inhibition is likely to be caused by the sample matrix. Thus, we recommend our ExtractNow™ Vegan Control kit for sample preparation. Any other DNA extraction kit needs to be qualified.

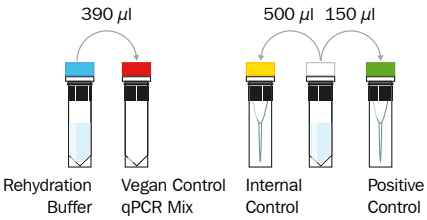
⇨ It is important to include control samples on a regular basis to monitor the reliability of your results. Positive and negative (non-template) controls are essential in case of troubleshooting and should be prepared in duplicates in each PCR. Use elution buffer for the NTC in case of extracted DNA.

⇨ The control samples must be processed in the same manner as the test samples. You may want to include other laboratory specific control samples such as high, median and low DNA levels.

PROCEDURE – OVERVIEW

1. Reagent Preparation

Vegan Control qPCR Mix
Positive Control
Internal Control



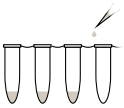
for 5 min RT
briefly
for 5 sec

2. Reaction Mix Preparation !

1 reaction
15 µl Vegan Control qPCR Mix (red cap)
1 µl Internal Control (yellow cap)

3. Loading the Test Tubes

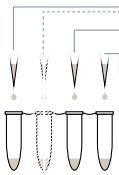
aliquot 15 µl Reaction Mix



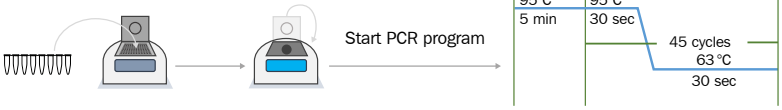
4. Adding Samples

+ 5 µl DNA extract
+ 5 µl Positive Control (green cap)
+ 5 µl Elution Buffer (Negative Control)

briefly



5. PCR Amplification



! If Internal Control was already added during DNA extraction, skip step 2 and proceed directly to step 3 and aliquot 15 µl Vegan Control qPCR Mix (red cap).

Rehydration Buffer
Vegan Control qPCR Mix
PCR grade water
Positive Control
Internal Control

incubate
vortex
centrifuge
add

storage 2-8 °C
after rehydration ≤ -18 °C

This procedure overview is not a substitute for the detailed manual.

PROCEDURE - STEP BY STEP

All reagents and samples must be equilibrated to +2 to +8 °C prior use. After reconstitution, the reagents should be stored at ≤ -18 °C. Repeated freezing and thawing should be avoided and reconstituted controls should be stored in aliquots.

1. Reagents preparation

1.	Vegan Control qPCR Mix Positive Control DNA Internal Control DNA	red cap green cap yellow cap	Spin down lyophilized components at max speed for 5 sec.
2.	Vegan Control qPCR Mix	red cap	Add 390 µl Rehydration Buffer (blue cap).
3.	Positive Control DNA	green cap	Add 150 µl PCR grade Water (white cap).
4.	Internal Control DNA	yellow cap	Add 500 µl PCR grade Water (white cap).
5.	Vegan Control qPCR Mix Positive Control DNA Internal Control DNA	red cap green cap yellow cap	Incubate at room temperature for 5 min.
6.	Vegan Control qPCR Mix Positive Control DNA Internal Control DNA	red cap green cap yellow cap	Vortex briefly and spin down for 5 sec.

2. Reaction mix preparation

The following steps (2. Reaction mix preparation, 3. Addition of samples and controls, and 4. PCR amplification) should be performed within 45 minutes to avoid a significant reduction of the fluorescent signal.

Optional: To assess the performance of the DNA extraction, the Internal Control DNA (at least 20 µl) can be added to the original sample prior to extraction. In this case, do not add further Internal Control DNA to the PCR reaction mix and proceed directly to step 2 and aliquot 15 µl of Vegan Control qPCR Mix (red cap) to each PCR reaction tube.

We recommend following strictly this protocol and pipetting sequence:

1.	Calculate the required volume of master mix for all the control and test reactions and prepare it as indicated below, at room temperature, and in a 1.5 ml reaction tube.			
		for 1 reaction	**for 25 reactions**	**cap color**
	Vegan Control qPCR Mix	15 µl	375 µl	red
	Internal Control DNA	1 µl	25 µl	yellow
2.	Mix by pipetting up and down (5 times).			
3.	Add 15 µl to each PCR tube and close the lid.			

3. Addition of samples and controls

Add samples according to the following pipetting sequence:

1.	Negative Controls: add 5 µl H_2O or elution buffer from DNA extraction kit.
2.	Samples: add 5 µl of extracted DNA from food sample.
3.	Positive Control: add 5 µl Positive Control DNA (green cap).
4.	Close the PCR tubes tightly and spin down briefly.

4. PCR amplification

1.	Place PCR tubes in the qPCR cycler and close the lid.
2.	Program the cycler as indicated below or see Appendix I for detailed cycler programs of selected qPCR cyclers. Programs for additional cyclers are available upon request. 1 cycle — 95 °C for 5 min 45 cycles — 95 °C for 30 sec (Denaturation) 63 °C for 30 sec (Annealing, elongation and data collection) **Fluorescent dyes: FAM™ and HEX™**
3.	Start the program.

This assay has been successfully performed on the following qPCR devices:

qPCR device	Manufacturer
CFX96 Touch™	Bio-Rad Laboratories
ABI Prism® 7500	Applied Biosystems
Rotor-Gene® 6000	Corbett Research
Mx3005P™	Agilent Technologies

DATA INTERPRETATION

The presence of unwanted meat- or animal-derived contaminants in food samples is indicated by an increasing fluorescence signal in the FAM™ channel.
We recommend evaluating the progression of the amplification curve for any sample, including the controls. The quantification is based on threshold cycle (Ct) values and a DNA standard curve run in parallel with the samples. The exact procedure to obtain the Ct values, perform baseline calculation/normalization, and threshold setting depends on the particular qPCR device and software in use. Please refer to the documentation of your specific device for further details.

A positive PCR result is indicated by a Ct $<$ 40. PCR reactions with Ct $\geq$ 40 are considered negative.
In addition, a successful PCR reaction (no inhibition) displays an increasing fluorescent signal in either the FAM™ and/or the HEX™ channel (for the Internal Control). The animal target DNA might compete with the Internal Control in the PCR. Thus, the higher the levels of animal-derived DNA in the sample, the higher the signal in the FAM™ channel and the lower the internal control signal in the HEX™ channel.

The following table will help with the interpretation of PCR results:

Detection of animal traces FAM™ channel	Internal control HEX™ channel	Interpretation
positive	irrelevant	animal traces detected
negative	negative	PCR inhibition
negative	positive	animal traces not detected

ASSAY CHARACTERISTICS

1. Sensitivity

The Vegan Control™ kit shows a PCR detection sensitivity of $\leq$ 10 pg/PCR of animal DNA. It is possible to detect animal-derived contaminants in a vegan food products down to a threshold level of 0.1 % when this kit is combined with ExtractNow™ Vegan Control. This DNA extraction kit was especially optimized for the extraction of DNA from different kinds of food samples.

2. Specificity

No cross-reactivity with non-animal DNA could be observed (e.g. plant-based protein substitutes as seitan or tofu etc.).

APPENDIX I

These protocols have been created based on internal experience and customer feedback to provide general support to the user in the setting of the qPCR instrument. Minerva Biolabs shall not warrant or assume any responsibility for the performance of these protocols.

Programming the LightCycler® 2.0

Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature [°C]	95
Incubation time [min]	5:00
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Program 2: Amplification

Cycles	45	
Analysis Mode	Quantification	
Temperature Targets	***Segment 1***	***Segment 2***
Target Temperature [°C]	95	63
Incubation time [s]	30	30
Temperature Transition Rate [°C/s]	20.0	20.0
Secondary Target Temperature [°C]	0	0
Step Size [°C]	0.0	0.0
Step Delay [Cycles]	0	0
Acquisition Mode	None	Single

Program 3: Cooling

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature [°C]	40
Incubation time [s]	30
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Result Reading:

- Select the fluorescence channels 1 and 2.
- Click on Quantification to generate amplification plots and Ct values.
- The threshold will be generated automatically.
- Samples showing no significant increase in the amplification plot can be considered as negative.

Programming the ABI Prism® 7500

Detector Settings:

Target Probe:	Reporter - FAM™ / Quencher - none
Internal Control Probe:	Reporter - HEX™ / Quencher - none

The “ROX Reference” function needs to be disabled, as no ROX™ dye is included in the mix.
Activate both detectors for each well.
Measure fluorescence during annealing.

Program Step 1: Pre-incubation

Setting	Hold
Temperature	95 °C
Incubation time	5:00 min

Program Step 2: Amplification

Cycles	45
Setting	Cycle
Denaturing	95 °C for 30 sec
Annealing	63 °C for 30 sec & data reading

Result Reading:

- Enter the following basic settings at the right task bar:
  Data: Delta RN vs. Cycle
  Detector: FAM™ and HEX™
  Line Color: Well Color
- Open a new window for the *Graph setting*s by clicking the right mouse button
  Select the following setting and confirm with *ok*:
  Real Time Settings: Linear
  Y-Axis Post Run Settings: Linear and Auto Scale
  X-Axis Post Run Settings: Auto Scale
  Display Options: 2
- Initiate the calculation of the C_t values and the graph generation by clicking on *Analyze* within the report window.
- Pull the threshold line into the graph. Adapt the threshold line to the initial linear section obtained for the positive control reaction.
- Samples showing no C_t value can be considered as negative.

Programming the Rotor-Gene® 6000 (5-plex)

Please check the correct settings for the filter combination:

Target	Animal DNA	Internal Control DNA
Filter	green	yellow
Wavelenght	510 nm	555 nm

Program Step 1: Pre-incubation

Setting	Hold
Hold Temperature	95 °C
Hold Time	5 min 0 sec

Program Step 2: Amplification

Setting	Cycling
Cycles	45
Denaturation	95 °C for 30 sec
Annealing	63 °C for 30 sec → **acquiring to Cycling A (green and yellow)**
Gain setting	automatic (auto Gain)
Slope Correct	activated
Ignore First	deactivated

Result Reading:

- Open the menu *Analysis*
- Select *Quantitation*
- Check the required filter set (green and yellow) according to the following table and start data analysis by double click.
- The following windows will appear:

  Quantitation Analysis - Cycling A (green or yellow)
  Quant. Results - Cycling A (green or yellow)
  Standard Curve - Cycling A (green or yellow)

- In window *Quantitation Analysis*, select first linear scale and then *slope correct*
  Threshold setup (not applicable if a standard curve was included in parallel and auto threshold was selected)
  - In window *CT Calculation* set the threshold value to 0-1
  - Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- The Ct values can be taken from the window Quant. Results.

Programming the Mx3005P™

- Go to the setup menu, click on *„Plate Setup“,* check all positions that apply
- Click on *„Collect Fluorescence Data“* and check FAM™ and HEX™
- According to the basic settings, the *„Reference Dye“* function should be deactivated
- Specify the type of sample (negative or positive control, sample, standard) at *„well type“*
- Edit the temperature profile at *„Thermal Profile Design“:*

Segment 1:	95 °C for 5 min
Segment 2:	
Denaturing	95 °C for 30 sec
Annealing	63 °C for 30 sec & data collection end
45 cycles	

- at menu *„Run Status“* select *„Run“* and start the cycler by pressing *„Start“*

Analysis of raw data:

- In the window *„Analysis“,* tap on *„Analysis Selection / Setup“* to analyze the marked positions
- Ensure that in window *„Algorithm Enhancement“* all options are activated:
  Amplification-based threshold
  Adaptive baseline
  Moving average
- Click on *„Results“* and *„Amplification Plots“* for automatic thresholding
- Read the C_t values in *„Text Report“*

Programming the LightCycler® 480

Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature [°C]	95
Incubation time [min]	5:00
Temperature Transition Rate [°C/s]	4.4
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Program 2: Amplification

Cycles	45	
Analysis Mode	Quantification	
Temperature Targets	***Segment 1***	***Segment 2***
Target Temperature [°C]	95	63
Incubation time [s]	30	30
Temperature Transition Rate [°C/s]	4.4	2.2
Secondary Target Temperature [°C]	0	0
Step Size [°C]	0.0	0.0
Step Delay [Cycles]	0	0
Acquisition Mode	None	Single

Program 3: Cooling

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature [°C]	40
Incubation time [s]	30
Temperature Transition Rate [°C/s]	2.2
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Before starting the LC480, make sure that the filter settings are correct:

LightCycler® 480	Animal Traces	Internal Control
Instrument I	533 nm	568 nm
Instrument II	510 nm	580 nm

Programming the CFX 96 Touch™, CFX96 Touch Deep Well™, CFX Connect™, and CFX384 Touch™ (Bio-Rad)

Performing Runs

Run Setup - Protocol Tab

- Click **Create New** to open the Protocol Editor to create a new protocol.
- Select any step in either the graphical or text display. The selected step becomes highlighted in blue. Click the temperature or incubation time to directly edit the value.

	Step 1	Step 2	Step 3	Step 4
Temperature	95.0 ℃	95.0 ℃	63.0 ℃	GO TO STEP 2
Incubation time	05:00 min	00:30 sec	00:30 sec	× 45

Run Setup - Plate Tab

- Click **Create New** to open the Plate Editor to create a new plate.
- Use the **Scan Mode** dropdown menu in the Plate Editor toolbar to designate the data acquisition mode to be used during the run. Important!!! Select the **All Channels** mode.
- Click the **Select Fluorophores** button to indicate the fluorophores that will be used in the run.
- Select the wells to be loaded within the plate diagram.

Run Setup – Start Run Tab

- View the selected Protocol file, Plate file, and data acquisition Scan Mode setting in the **Run Information** panel.
- Select one or more blocks and edit run parameters if necessary in Start Run on Selected Block(s) pane.
- Click the Start Run button to begin the run.

Data Analysis

Quantification Tab

The amplification chart data in this tab display the fluorescence values as relative fluorescence units (RFU) collected from each well at every cycle of the run.

- Choose the fluorophore data you want to display by ticking the checkboxes corresponding to the fluorophore of interest located under the amplification chart.

Data Analysis Settings

- The software uses two modes for quantification cycle determination. Select **Settings** from the menu bar and select **Baseline Subtracted Curve Fit** as baseline setting and **Single Threshold Mode** as Cq Determination Mode.

- In the **Single Threshold Mode**, click and manually drag the threshold line to position it where appropriate. Adapt the threshold line to the initial linear section of the positive control reaction.

- Samples showing no Ct value can be considered as negative.

APPENDIX II

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from of the use, the results of use, or the inability to use this product.

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Related Products

qPCR Kits for Meat Identification		
370-1025/-1100	Meat ID™ Halal	25/100 reactions
qPCR Kits for Food Contamination Testing		
36X-X025	Food Control™ qPCR	25 reactions
qPCR Kits for Water Contamination Testing		
33-2025/-2100/-2250	AquaScreen® Legionella species	25/100/250 reactions
34-2025/-2100/-2250	AquaScreen® Legionella pneumophila	25/100/250 reactions
34-6025/-6100/-6250	AquaScreen® Pseudomonas aeruginosa	25/100/250 reactions
34-7025/-7100/-7250	AquaScreen® Escherichia coli	25/100/250 reactions
DNA Extraction kits		
609-1010/1050	ExtractNow™ Food Control	
56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
601-1010/1050/1200	ExtractNow™ DNA Mini Kit	10/50/200 extractions
602-1010/1050/1200	ExtractNow™ Blood DNA Mini kit	10/50/200 extractions
603-1010/1050/1200	ExtractNow™ RNA Mini kit	10/50/200 extractions
604-1010/1050/1200	ExtractNow™ Cleanup kit	10/50/200 extractions
605-1010/1050/1200	ExtractNow™ Plasmid Mini kit	10/50/200 extractions
606-1010/1050/1200	ExtractNow™ Virus DNA/RNA kit	10/50/200 extractions
607-1010/1050	ExtractNow™ Vegan Control	10/50 extractions
608-1010/1050	ExtractNow™ Meat ID	10/50 extractions
PCR Cycler Validation		
57-2102	PCR Cycler Check™ Advance	6 strips, 8 vials each
57-2103	PCR Cycler Check™ OneStep	100 reactions
57-2202	qPCR Cycler Check™	100 reactions
Lab Monitoring Kits		
181-0010/-0050	SwabUp™ Lab Monitoring, For sample collection and DNA extraction	10/50 samples
PCR Mix		
191-0025/-0100/-0250	ConviFlex™ DNAmp Mix, PCR Mix with Taq polymerase for conventional and qPCR	25/100/250 reactions
192-0025/-0100/-0250	ConviFlex™ RT-Taq Mix, RT-PCR Mix with Taq polymerase and retrotrascriptase for conventional and RT-qPCR	25/100/250 reactions
PCR Clean™		
15-2025/-2200	DNA Decontamination Reagent, spray bottle/refill canister	250 ml/4×500 ml
15-2500	DNA Decontamination Reagent, refill canister	5 l
15-2001	DNA Decontamination Reagent, Wipes in dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes, refill pack	5×50 wipes
LabClean™		
15-4100	Molecular Microbiology Lab Cleaner	1 liter
Mycoplasma Off™		
15-1000/-5000	Surface Disinfectant Spray, spray bottle, refill canister	1 l/5 l
15-1001	Surface Disinfectant Wipes in dispenser box	50 wipes
15-5001	Surface Disinfectant Wipes in refill pack	5×50 wipes
WaterShield™		
15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200× concentrate	15×10 ml/3×50 ml/500 ml

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