THP1-Dual™ KI-hSTING-H232 Cells

STING (H232 isoform) knockin NF-κB-SEAP and IRF-Lucia Reporter Monocytes

Catalog code: thpd-h232

http://www.invivogen.com/thp1-dual-ki-hsting-h232

For research use only

Version 20J26-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of THP1-Dual™ KI-hSTING-H232 cells** (3-7 x 10^6 cells)
- **1 ml of Blasticidin** (10 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of Zeocin™** (100 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

- **1 pouch of QUANTI-Luc™** (Lucia luciferase detection medium) Store QUANTI-Luc™ pouch at -20 °C for 12 months. Reconstituted QUANTI-Luc™ medium is stable for 1 week at 4 °C and for 1 month at -20 °C. Protect QUANTI-Luc™ from light.
- **1 ml of QB reagent** and **1 ml of QB buffer** (sufficient to prepare 100 ml of **QUANTI-Blue™ Solution**, a SEAP detection reagent). Store QB reagent and QB buffer at -20 °C. QUANTI-Blue™ Solution is stable for 2 weeks at 4 °C and for 2 months at -20 °C.

Note: Data sheets for all components are available on our website.

Handling Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

*Note: **Avoid freezing cells upon receipt** as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

Cell Line Stability

Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Quality Control

- The knockin (KI) of the human STING variant (H232) has been verified by functional assays, PCR and sequencing.
- Reporter activity has been validated using functional assays.
- Stability for 20 passages following thawing has been verified.
- The cell line is guaranteed mycoplasma-free.

USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product the buyer agrees the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

BACKGROUND

STING (stimulator of interferon genes) is essential for the interferon (IFN) response to cytoplasmic foreign or self-DNA. It directly senses cyclic dinucleotides (CDNs), which are important messengers in bacteria and innate immune agonists in mammals[1]. Distinct variants of human STING (hSTING) that affect CDN recognition and signal transduction have been identified:

- **R232** (R71-G230-**R232**-R293): the most prevalent in the human population (~60%). Referred as the "wild-type" or 232R-RGR allele[2].
- **HAQ** (**H71**-**A230**-R232-**Q293**): contains three non-synonymous single nucleotide substitutions; R71H, G230A and R293Q. This allele, found in ~20% of the population, is less sensitive to CDNs than the "wild-type" allele[2].
- **H232** (R71-G230-**H232**-R293): the most commonly used hSTING variant in structural studies[2].
- **S154**: a gain-of-function mutation resulting in constitutive STING activation[3].
- **M155**: a gain-of-function mutation resulting in constitutive STING activation[3,4].
- **A162**: a synthetic mutation (S162A) that confers hSTING sensitivity to DMXAA, a tumor vascular disrupting agent in mice[5].

THP1-Dual™ KI-STING cells are a family of reporter cells allowing the study of STING variation by monitoring the activation of the transcription factors ISRE (IFN-stimulated response elements) and NF-κB. They were generated from THP1-Dual™ KO-STING cells, which derive from the human THP-1 monocytes, by stable biallelic knockout of the endogenous HAQ hSTING gene and stable integration of two inducible secreted reporter genes: Lucia luciferase and SEAP (secreted embryonic alkaline phosphatase). The Lucia luciferase gene is under the control of an ISG54 (interferon-stimulated gene) minimal promoter in conjunction with five ISRE. The SEAP gene is driven by an IFN-β minimal promoter fused to five copies of the NF-κB response element. As a result, they allow the simultaneous study of the IFN regulatory factor (IRF) and NF-κB pathways. Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Luc™, a Lucia™ detection reagent, and QUANTI-Blue™, a SEAP detection reagent.

CELL LINE DESCRIPTION

THP1-Dual™ KI-hSTING-H232 cells were generated from THP1-Dual™ KO-STING cells by knockin of the intronless coding sequence (from ATG to TGA) of the H232 hSTING variant. R71-G230-**H232**-R293 has been identified as a natural variant allele of STING occurring in ~14% of the human population[2]. The H232 isoform contains the single amino acid substitution R232H. This isoform is associated with a diminished response to bacterial and metazoan CDNs when compared to the prevalent R232 hSTING allele[2,6]. THP1-Dual™ KI-hSTING-H232 cells are resistant to Blasticidin and Zeocin™.

1. Sun L. *et al.*, 2013. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science. 339:786-91. **2. Yi G. *et al.*, 2013.** Single nucleotide polymorphisms of human STING can affect innate immune response to cyclic dinucleotides. PLOS One. 8:e77846. **3. Liu Y. *et al.*, 2014.** Activated STING in a vascular and pulmonary syndrome. N Engl J Med. 371:507-18. **4. Jeremiah N. *et al.*, 2013.** Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. J Clin Invest. 124:5516-20. **5. Gao P. *et al.*, 2014.** Binding-pocket and lid-region substitutions render human STING sensitive to the species-specific drug DMXAA. Cell Reports. 8:1668-76. **6. Diner E. *et al.*, 2013.** The innate immune DNA sensor cGAS produces a noncanonical cyclic dinucleotide that activates human STING. Cell Rep 3(5):1355-61.

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FAQ Any questions about our cell lines? Visit our FAQ page.

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

• **Growth Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated fetal bovine serum (FBS; 30 min at 56°C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)

Initial culture of all THP-1 derived cells must be performed in growth medium containing 20% heat-inactivated FBS.

Note: The use of Normocin™ together with Pen-Strep is required to keep the cells free of microbial contaminants. Contamination of this cell line may activate TLRs resulting in differentiation of the monocytes and activation of the reporter gene.

• **Freezing Medium:** 90% fetal bovine serum (FBS), 10% DMSO

• **Test Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/ml-100 µg/ml).

Required Selective Antibiotics

Blasticidin and Zeocin™

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

Note: All steps from this point should be carried out under aseptic conditions.

3. Transfer cells in a vial containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS). **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge cells at 150 x g (RCF) for 10 mins.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS).
6. Transfer the vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
7. Place the culture at 37°C, 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freshly prepared freezing medium.
2. Dispense 1 ml of the cell suspension into cryogenic vials.
3. Place vials in a freezing container and store at -80°C overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell Maintenance

1. After cells have recovered (after at least two passages), subculture the cells in growth medium (with 10% heat-inactivated FBS). To maintain selection pressure, add 10 µg/ml of Blasticidin and 100 µg/ml of Zeocin™ to the growth medium every other passage.
2. Pass the cells every 3 days by inoculating 5 x 10^5 cells/ml. Do not allow the cell concentration to exceed 2 x 10^6 cells/ml.

Cell Handling Recommendations

To ensure the best results:

- ***Use THP1-Dual™ KI-hSTING-H232 cells with less than 20 passages.***
- *Handling of cells should be as short as possible to prevent any damage resulting from the prolonged stay at room temperature without 5% CO_2.*

CELL PREPARATION

Use THP1-Dual™ KI-hSTING-H232 cells with THP1-Dual™ KO-STING cells (negative control) and THP1-Dual™ cells or THP1-Dual™ KI-hSTING-R232 (positive control).

1. Centrifuge at 150 x g (RCF) for 10 mins or 300 x g (RCF) for 5 mins.
2. Remove supernatant and resuspend THP1-Dual™ KI-hSTING-H232 cells at 5 x 10^5 cells/ml in freshly prepared test medium.

Note: It is recommended to perform the assay with test medium which does not contain Normocin™ nor Zeocin™.

Induction of THP1-Dual™ KI-hSTING-H232 Cells

Day 1:

1. Add 20 µl of each test coumpound per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control such as 2'3'cGAMP at 30 µg/ml in one well.
3. Add 20 µl of a negative control such as sterile, endotoxin-free water in another well.
4. Prepare a cell suspension of THP1-Dual™ KI-hSTING-H232 cells at ~500,000 cells per ml in test medium.
5. Add 180 µl of cell suspension (~100,000 cells) per well.
6. Incubate the plate at 37 °C in a CO_2 incubator for 20-24 h.

Detection of the NF-κB response using QUANTI-Blue™

Day 2:

1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed technical data sheet.
2. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a new flat-bottom 96-well plate.
3. Add 20 µl of induced THP1-Dual™ KI-hSTING-H232 cell culture supernatant.
4. Incubate the plate at 37 °C incubator for 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Detection of the ISRE response using QUANTI-Luc™

Below is a protocol for end-point readings using a luminometer. It can be adapted for use with kinetic measurements.

Day 2:

1. Prepare QUANTI-Luc™ following the instructions on the enclosed technical data sheet.
2. Pipet 10 µl of THP1-Dual™ KI-hSTING-H232 cell culture supernatant per well in a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ per well.
4. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Catalog Code
2'3'-cGAMP	tlrl-nacga23
2'3'-cGAM(PS)2 (Rp/Sp)	tlrl-nacga2srs
3'3'-cGAMP Fluorinated	tlrl-nacgaf
Blasticidin	ant-bl-05
Normocin™	ant-nr-1
QUANTI-Blue™ Solution	rep-qbs1
QUANTI-Luc™ Gold	rep-qlcg1
THP1-Dual™	thpd-nfis
THP1-Dual™ KI-hSTING-A162	thpd-a162
THP1-Dual™ KI-hSTING-R232	thpd-r232
THP1-Dual™ KI-hSTING-M155	thpd-m155
THP1-Dual™ KI-hSTING-S154	thpd-s154
THP1-Dual™ KO-STING	thpd-kostg
Zeocin™	ant-zn-1

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QUANTI-Blue™ Solution

Medium for detection and quantification of alkaline phosphatase in standard and HTS assays

Catalog code: rep-qbs, rep-qbs2

http://www.invivogen.com/quanti-blue

For research use only

Version 18D13-MM

PRODUCT INFORMATION

Contents

QUANTI-Blue™ Solution is available in two pack sizes:

• **rep-qbs** containing 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer to prepare 500 ml of QUANTI-Blue™ Solution sufficient for 25 x 96-well plates (standard procedure) or 20 x 1536-well plates (HTS screening)

• **rep-qbs2** containing 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer to prepare 1 liter of QUANTI-Blue™ Solution sufficient for 50 x 96-well plates (standard procedure) or 40 x 1536-well plates (HTS screening)

Required Material (not provided)

• Sterile water

• Sterile screw cap tube, glass bottle or flask

Storage and Stability

• Store QB reagent and QB buffer at -20 °C. Product is stable for 1 year at -20 °C when properly stored.

• Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8 °C and for 2 months at -20 °C. Keep reconstituted QUANTI-Blue™ away from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

• Physicochemical characterization (including pH, solubility).

• Functional assays using alkaline phosphatase or SEAP-expressing reporter cells.

DESCRIPTION

QUANTI-Blue™ is a colorimetric enzyme assay developed to determine any alkaline phosphatase activity (AP) in a biological sample, such as supernatants of cell cultures. QUANTI-Blue™ Solution changes from pink to a purple-blue color in the presence of AP.

Secreted embryonic alkaline phosphatase (SEAP) is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a GPI-anchored protein. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters.

FEATURES AND ADVANTAGES

• **Requires small samples of cell supernatants** - 20 µl is sufficient.

• **No need to process samples** - Preparation of cell lysates or heating of samples is not required.

• **Determine secreted AP activity without disturbing cells** - The same cell cultures can be repeatedly sampled for kinetic studies.

• **Assay can be completed in 30 min** - Hands-on time no longer than 10 min. The enzymatic activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™ Solution.

• **Wide dynamic range allows to detect low and high levels of AP** - No need to perform multiple sample dilutions.

• **Highly sensitive for quantitative measurement** - Higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity.

• **Extremely simple to use** - 1) Prepare solution with water, 2) add sample to detection reagent, 3) incubate at 37 °C, and 4) assess AP activity.

METHODS

QUANTI-Blue™ Solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high throughput screening procedure).

A. Standard procedure

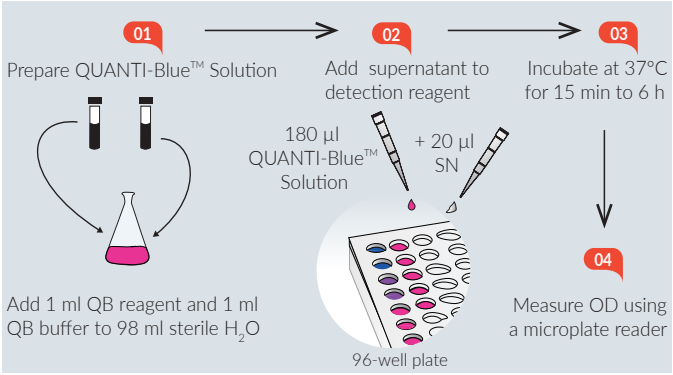

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

The following protocol refers to the use of 96-well plates. Ensure QB reagent and QB buffer are completely thawed before use.

*Note: For fast thawing, QB reagent and QB buffer can be placed at 37 °C for 2 minutes. Ensure heating at 37 °C does **not** exceed 5 minutes.*

1. Prepare 100 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water in a sterile glass bottle or flask.
2. Mix well by vortexing and incubate at room temperature for 10 min before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
4. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
6. Incubate at 37 °C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend to heat FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

For different cell culture plate formats, please refer to the table below:

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

TECHNICAL SUPPORT
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B. High Throughput Screening procedure

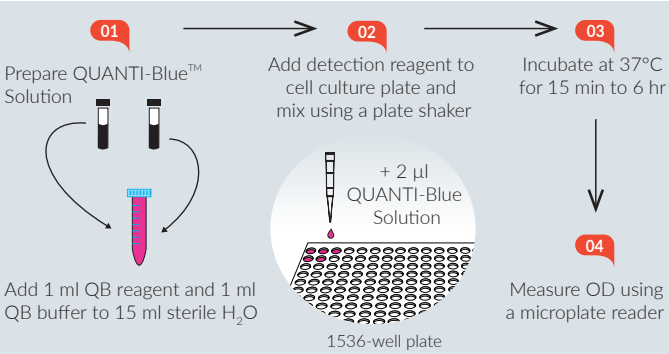

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

This procedure has been optimized for use directly in flat-bottom 1536-well plates, in which cell culture volume does not exceed **5 µl**. Ensure QB reagent and QB buffer are completely thawed before use.
*Note: For fast thawing, QB reagent and QB buffer can be placed at 37 °C for 2 minutes. Ensure heating at 37 °C does **not** exceed 5 minutes.*

1. Prepare 17 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 15 ml of sterile water in a 50 ml screw cap tube.
2. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
4. Dispense 2 µl of QUANTI-Blue™ Solution per well of a 1536-well plate.
5. Mix using a plate shaker.
6. Incubate at 37 °C for 15 min to 6 h.
7. Measure OD at 620-655 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend to heat FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo^R)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
Reporter cells	
HEK-Blue™ hTLR2	hkb-htlr2
HEK-Blue™ hTLR4	hkb-htlr4
RAW-Blue™ Cells	raw-sp
THP1-Blue™ NF-κB Cells	thp-nfkb
THP1-Blue™ ISG Cells	thp-isg

For a complete list of InvivoGen's Reporter Cell Lines visit
http://www.invivogen.com/reporter-cells

QUANTI-Luc™

A coelenterazine-based luminescence assay reagent

Catalog code: rep-qlc1, rep-qlc2

http://www.invivogen.com/quanti-luc

For research use only

Version 18D30-MM

PRODUCT INFORMATION

Contents

QUANTI-Luc™ is provided as packs of individually sealed pouches.

- rep-qlc1: 2 pouches of QUANTI-Luc™
- rep-qlc2: 5 pouches of QUANTI-Luc™

Each pouch contains everything needed to prepare 25 ml of reagent allowing the preparation of 500 wells of a 96-well plate.

Storage and Stability

- Store QUANTI-Luc™ pouches at -20°C for 12 months.
- Reconstituted QUANTI-Luc™ is stable for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: *This product is photosensitive and should be protected from light.*

DESCRIPTION

QUANTI-Luc™ is an assay reagent containing all the components required to quantitively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases. QUANTI-Luc™ contains the coelenterazine substrate and stabilizing agents for the luciferase reaction. The light signal produced is quantified using a luminometer and expressed as relative light units (RLU). The signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

QUANTI-Luc™ is optimized for use with Lucia luciferase reporter cell lines. Lucia luciferase is a secreted coelenterazine luciferase encoded by a synthetic gene. As Lucia luciferase is secreted, it can be directly measured in the cell culture medium using bioluminescent assays.

InvivoGen provides a recombinant Lucia luciferase protein (see Related Products) which is a positive control for QUANTI-Luc™. A dilution series of the recombinant Lucia luciferase protein can also be used to determine the linear range of the assay.

METHODS

Preparation of QUANTI-Luc™

1. Pour the pouch contents into a 50 ml screw cap tube.
2. Add 25 ml of sterile water.
3. Swirl product gently until powder is completely dissolved.
4. Use QUANTI-Luc™ assay solution immediately or store until required for use. Reconstituted QUANTI-Luc™ can be stored for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: *This product is photosensitive and should be protected from light.*

Detection of luciferase activity from cell culture medium

To obtain **end-point readings** using a luminometer **with an injector**.

1. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with the QUANTI-Luc™ assay solution and proceed **immediately** with the measurement.

To obtain **end-point readings** using a luminometer **without injectors**.

1. Set the luminometer with a 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ assay solution to each well or tube.
4. Gently tap the plate several times to mix (do **not** vortex).
5. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™ Gold (For standard and HTS assays)	rep-qlcg1
pSelect-zeo-Lucia™ (expression plasmid)	psetz-lucia
Recombinant Lucia™ protein	rec-lucia
Reporter Cells	
THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells	thpd-nfis
THP1-Lucia™ NF-κB Cells	thp1-nfkb

For a complete list of InvivoGen's Lucia luciferase Reporter Cell Lines visit http://www.invivogen.com/lucia-reporter-cells

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