

# HEK-Dual™ hTLR5 (NF/IL8) Cells (NF-κB-SEAP/KI-[IL-8]Lucia)

Dual NF-κB and IL-8 reporter HEK293 cells expressing human TLR5

Catalog code: hkd-htlr5ni

<https://www.invivogen.com/hek-dual-htlr5>

For research use only

Version 21F18-MM

## PRODUCT INFORMATION

### Content

- 1 vial of HEK-Dual™ hTLR5 (NF/IL8) Cells (3-7 x 10<sup>6</sup> cells)
- 1 ml of Hygromycin B Gold (100 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), a formulation of 3 antibiotics

active against mycoplasmas, bacteria and fungi. Store at -20°C.\*

\*The expiry date is specified on the product label.

- 1 ml of QB reagent and 1 ml of QB buffer (sufficient to prepare 100 ml of QUANTI-Blue™ Solution, a SEAP detection reagent). QB reagent and QB buffer are stable for 1 year at -20°C. QUANTI-Blue™ Solution is stable for 2 weeks at 4°C and for 2 months at -20°C.

- 1 pouch of QUANTI-Luc™. Store at -20°C for 12 months. Reconstituted medium is stable for 1 week at 4°C and 1 month at -20°C. Protect from light.

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

### Handling Cells Upon Receipt

Cells must be thawed **immediately** upon receipt and grown according to handling procedures (see next page), to ensure cell viability and proper assay performance.

*Note: Do not freeze the 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

Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. To ensure maximum efficiency, do not passage HEK-Dual™ hTLR5 (NF/IL8) cells more than 20 times.

### Quality Control

- The biallelic replacement of the human interleukin-8 (IL-8) open reading frame (ORF) with the Lucia luciferase reporter ORF was verified by PCR and sequencing. Furthermore, the inability to produce IL-8 has been confirmed by ELISA.
- TLR3 and TNF receptor (TNFR) knockout has been verified by functional assays and DNA sequencing to confirm frameshift deletion.
- The expression of the human TLR5 (hTLR5) gene has been confirmed by RT-PCR.
- The response to various TLR agonists has been tested. As expected, only TLR5 agonists induced the production of the reporter proteins.
- The cell line stability for 20 passages following thawing has been verified.
- The cell line is guaranteed mycoplasma-free.

## BACKGROUND

HEK-Dual™ TLR (NF/IL8) cells are a family of Toll-like receptor (TLR)-reporter cells designed for the study of the inflammatory response induced by the stimulation of a given TLR by monitoring the activation of the transcription factors NF-κB and AP-1 and/or the expression of interleukin-8 (IL-8). IL-8 is a chemokine produced in response to TLR agonists in an NF-κB and AP-1 dependent-manner<sup>1,2</sup>. HEK-Dual™ TLR (NF/IL8) cells were generated from the human embryonic kidney 293 (HEK293)-derived cell line, HEK-Dual™ Null (NF/IL8), by stable transfection of a TLR gene. This parental cell line, that features a triple knockout of TLR3, TLR5 and TNFR (all of which are endogenously expressed in HEK293 cells), stably expresses an NF-κB/AP-1 inducible secreted embryonic alkaline phosphatase (SEAP) reporter construct. It also expresses Lucia luciferase, a secreted luciferase, placed under the control of the endogenous IL-8 promoter; the coding sequence of IL-8 was replaced by the Lucia luciferase ORF using knockin technology. Thus, TLR stimulation can be assessed in HEK-Dual™ TLR (NF/IL8) cells by monitoring NF-κB/AP-1-induced SEAP production and/or IL-8-dependent expression of Lucia luciferase.

The two reporter proteins, SEAP and Lucia Luciferase, can be readily measured in the supernatant by using QUANTI-Blue™ Solution and QUANTI-Luc™, respectively.

1. Ohta K. *et al.*, 2014. Toll-like receptor (TLR) expression and TLR-mediated interleukin-8 production by human submandibular gland epithelial cells. *Mol Med Rep.* 10(5):2377-82. 2. Roebuck KA. *et al.*, 1999. Regulation of interleukin-8 gene expression. *J Interferon Cytokine Res.* 19(5):429-38.

## CELL LINE DESCRIPTION

HEK-Dual™ TLR5 (NF/IL8) cells were generated from HEK-Dual™ Null cells by stable transfection of the human TLR5 (hTLR5) gene. Due to the knockout of TLR3, these cells enable the study of hTLR5 signaling without interference from other TLRs. They respond to low concentrations of TLR5 agonist flagellin. They do not respond to other TLR agonists or to the cytokine TNF-α (see validation sheet).

*Note: HEK-Dual™ hTLR5 (NF/IL8) and their parental cell line endogenously express NOD1.*

HEK-Dual™ hTLR5 (NF/IL8) cells are resistant to hygromycin B, Zeocin™ and blasticidin. They should be maintained in growth medium (see next page) supplemented with hygromycin B and Zeocin™.

## TECHNICAL SUPPORT

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## SAFETY CONSIDERATIONS

### Biosafety Level 2

HEK-Dual™ hTLR5 (NF/IL8) cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) and thus may require Biosafety Level 2. The biosafety level varies by country. In the United States, HEK293 cell lines are designated Biosafety Level 2 according to the Center for Disease Control and Prevention (CDC). In Germany, HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

## HANDLING PROCEDURES

### Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 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)
- **Freezing Medium:** DMEM, 20% (v/v) FBS, 10% (v/v) DMSO
- **Test Medium:** DMEM 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Hygromycin B Gold, Normocin™ and Zeocin™**

### Required Selective Antibiotics

Hygromycin B Gold 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% (v/v) ethanol.

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

3. Transfer cells into a larger vial containing 15 ml of pre-warmed growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 300 x g (RCF) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6. Transfer the vial contents to a 25 cm<sup>2</sup> tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the flask containing cells at 37 °C in 5% CO<sub>2</sub>.

### Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10<sup>6</sup> cells/ml in freezing medium freshly prepared with cold growth medium.

*Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.*

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 one passage), subculture the cells in growth medium supplemented with 100 µg/ml of Hygromycin B Gold and 50 µg/ml of Zeocin™.
2. Renew growth medium twice a week. Cells should be passaged when a 70-80% confluency is reached, detach the cells in the presence of PBS by tapping the flask or by using a cell scraper. Do not let the cells grow to 100% confluency.

*Note: The response of HEK-Dual™ hTLR5 cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Dual™ hTLR5 cells.*

### Induction of HEK-Dual™ hTLR5 (NF/IL8) Cells

#### Day 1:

1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control such as FLA-ST (flagellin from *Salmonella typhimurium*) at 100 ng/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 HEK-Dual™ hTLR5 (NF/IL8) cells at ~280,000 cells per ml in freshly prepared, pre-warmed test medium.
5. Add 180 µl of cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37 °C in a CO<sub>2</sub> incubator for 20-24 h.

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

#### Day 2:

1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed technical data sheet (TDS).
2. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
3. Add 20 µl of induced HEK-Dual™ hTLR5 (NF/IL8) 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 IL-8 response using QUANTI-Luc™

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

#### Day 2:

1. Prepare QUANTI-Luc™ following the instructions on the enclosed TDS.
2. Pipet 10 µl of HEK-Dual™ hTLR5 (NF/IL8) 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.

## 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 to 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.

## RELATED PRODUCTS

Product	Description	Cat. Code
FLA-ST	TLR5 ligand	tlrl-stfla
HEK-Dual™ Null Cells	Parental cells	hkd-null
Hygromycin B Gold	Selection antibiotic	ant-hg-1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
RecFLA-ST	TLR5 ligand	tlrl-flic
Zeocin™	Selection antibiotic	ant-zn-1

### TECHNICAL SUPPORT

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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, rep-qbs3

<https://www.invivogen.com/quant-blue>

For research use only

Version 20C16-MM

## PRODUCT INFORMATION

**Contents:** QUANTI-Blue™ Solution is available in three pack sizes

- **rep-qbs:** 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **25 x 96-well plates** (500 ml using the standard procedure) or **20 x 1536-well plates** (85 ml using the HTS screening procedure).

- **rep-qbs2:** 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **50 x 96-well plates** (1 L using the standard procedure) or **40 x 1536-well plates** (170 ml using the HTS screening procedure).

- **rep-qbs3:** 1 x 20 ml bottle of QB reagent and 1 x 20 ml bottle of QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **100 x 96-well plates** (2 L using the standard procedure) or **80 x 1536-well plates** (340 ml using the HTS screening procedure).

**Required Material (not provided)**

- Sterile water
- Sterile screw cap tube, glass bottle or flask

**Storage and stability**

- Product is shipped at room temperature. Upon receipt, store QB reagent and QB buffer at -20°C. Product is stable for 1 year at -20°C when properly stored.

- The 20 ml bottles of QB reagent and QB buffer are designed for single use. If required, individual aliquots of QB reagent and QB buffer can be prepared upon receipt or following a single freeze-thaw cycle. Store aliquots at -20°C. **Avoid repeated freeze-thaw cycles.**

*Note:* During storage, a precipitate may form in the 20 ml bottle of QB reagent. If this occurs, vortex the product until the precipitate disappears. The formation of a precipitate does not affect the activity of the product.

- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8°C and for 2 months at -20°C. Protect QUANTI-Blue™ 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 glycosylphosphatidylinositol (GPI)-anchored protein. SEAP is secreted into the cell culture supernatant and therefore offers many advantages over intracellular reporters.

QUANTI-Blue™ is highly sensitive for quantitative measurement. It has a higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity. Another advantage of QUANTI-Blue™ is that it can determine secreted AP activity without disturbing cells, thus allowing the repeated sampling of cell cultures for kinetic studies.

## TECHNICAL SUPPORT

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## 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

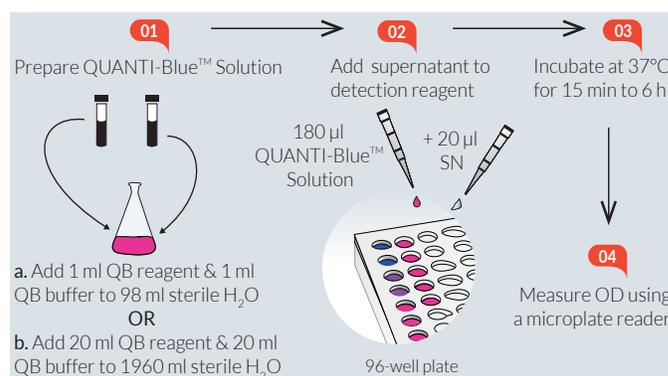


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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.

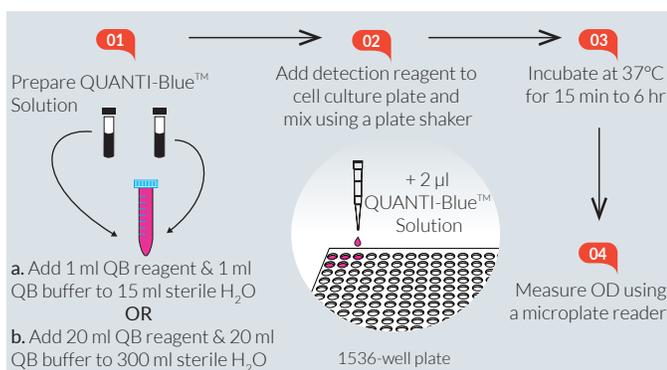
1. In a sterile bottle or flask, prepare QUANTI-Blue™ Solution by adding:
  - a. 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water.
  - b. 20 ml of QB reagent and 20 ml of QB buffer to 1960 ml of sterile water.
2. Mix by vortexing and incubate at room temperature for 10 min before use.
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 the 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 heating 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

## B. High Throughput Screening (HTS) procedure



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

This procedure has been optimized for use in HTS screening procedures in 1536-well plates. QUANTI-Blue™ Solution is added directly to the cell suspension to reduce liquid handling.

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. Dispense cell suspension and test compounds into a 1536-well plate in a volume that does not exceed **5 µl** per well. Incubate cells with test compounds for the desired period of time.
2. Prepare QUANTI-Blue™ Solution by adding:
  - a. **1 ml** of QB reagent and **1 ml** of QB buffer to **15 ml** of sterile water in a sterile 50 ml screw cap tube.
  - b. **20 ml** of QB reagent and **20 ml** of QB buffer to **300 ml** of sterile water in a sterile glass bottle or flask.
3. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
4. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
5. Dispense **2 µl** of QUANTI-Blue™ Solution to the wells containing  $\leq 5 \mu\text{l}$  of cell culture in a 1536-well plate.
6. Mix using a plate shaker.
7. Incubate at 37°C for 15 min to 6 h.
8. Measure OD at 620-655 nm.

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

## RELATED PRODUCTS

Product	Catalog Code
pNifTy2-SEAP (Zeo®)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
<b>Reporter cells</b>	
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 <https://www.invivogen.com/reporter-cells>

### TECHNICAL SUPPORT

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# QUANTI-Luc™

A coelenterazine-based luminescence assay reagent

Catalog code: rep-qlc1, rep-qlc2

<https://www.invivogen.com/quantiluc>

For research use only

Version 19A04-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 quantitatively 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 luciferase protein	rec-lucia
<b>Reporter Cells</b>	
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 <https://www.invivogen.com/lucia-reporter-cells>.

## TECHNICAL SUPPORT

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