THP1-Dual™ KO-cGAS Cells

cGAS knockout NF-κB-SEAP and IRF-Lucia luciferase reporter monocytes

Catalog # thpd-kocgas

http://www.invivogen.com/thp1-dual-kocgas

For research use only
Version # 17F28-MM

PRODUCT INFORMATION

Contents and Storage
- 1 vial of THP1-Dual™ KO-cGAS cells (3-7 x 10⁶ cells) in freezing medium

IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.
- 100 µl Blasticidin (10 mg/ml).
- 100 µl Zeocin™ (100 mg/ml).
- 1 ml 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-Blue™ (SEAP detection medium).
- 1 pouch of QUANTI-Luc™ (SEAP detection medium).

Handling Cells Upon Arrival
Cells must be thawed immediately upon receipt and grown according to handling procedures to ensure the best cell viability and assay performance. If you are unable to thaw the cells immediately, frozen cells may be placed in liquid nitrogen until you are ready to thaw and propagate them, however, this may reduce cell viability.

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. THP1-Dual™ KO-cGAS cells should not be passaged more than 20 times to remain fully efficient.

Quality control
• Biallelic cGAS knockout is verified by functional assays (see validation sheet), PCR and DNA sequencing.
• These cells are 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.

INTRODUCTION

Cyclic GMP-AMP synthase (cGAS, cGAMP synthase) is a critical cytosolic DNA sensor that triggers innate immune responses through the production of type I interferons (IFNs).1 In response to cytosolic double-stranded DNA (dsDNA), cGAS produces the cyclic dinucleotide (CDN) 2’3’-cGAMP. CDNs bind directly to STING, leading to TBK1-IRF3-mediated activation of IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG). The most potent agonist of human STING is 2’3’-cGAMP2, 3.

PRODUCT DESCRIPTION
THP1-Dual™ KO-cGAS cells were generated from THP1-Dual™ cells by stable knockout of the cGAS gene. They derive from human THP-1 monocytes, a cell line often used to study DNA sensing pathways as they express all the cytosolic DNA sensors identified so far (with the exception of DAI). THP1-Dual™ and THP1-Dual™ KO-cGAS cells stably express two inducible secreted reporter genes: Lucia luciferase and SEAP (secreted embryonic alkaline phosphatase). The Lucia luciferase reporter gene is under the control of an ISG54 (interferon-stimulated gene) minimal promoter in conjunction with five IFN-stimulated response elements. 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 NF-κB and IFN-β response elements.

SAFETY CONSIDERATIONS
Biosafety Level: 1

HANDLING PROCEDURES
Required Cell Culture Media
• 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)

Notes:
- 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.
- Heat-inactivated FBS is also commercially available.

Required Selective Antibiotic(s)
• 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 (approximately 2 minutes).
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 of the operations from this point should be carried out under strict aseptic conditions.
3. Transfer cells in 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 1000-1200 RPM (RCF = 200-300 g) 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 T-25 tissue culture flask containing 5 ml of growth medium.
7. Place the culture at 37°C in 5% CO2.

Frozen Stock Preparation
1. Resuspend cells at a density of 5-7 x 10⁶ cells/ml in freezing medium.
2. Aliquot 1 ml cells 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.
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 T-25 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the culture at 37°C in 5% CO2.

DETECTION OF NF-κB ACTIVATION
1. Add 20 µl of sample per well including a positive control (such as recombinant human TNF-α at 1 ng/ml) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
2. Add 180 µl of cell suspension (∼100,000 cells) per well of a flat-bottom 96-well plate.
3. Incubate the plate for 18-24 h at 37°C, 5% CO2.
4. Prepare QUANTI-Blue™ following the instructions on the enclosed data sheet.
5. Add 180 µl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.
6. Add 20 µl of THP1-Dual™ KO-cGAS cells supernatant.
7. Incubate the plate at 37°C for 1-6 h.
8. Determine SEAP levels using a spectrophotometer at 620-655 nm.

DETECTION OF IFN INDUCTION
Below is a protocol for end-point readings using a luminometer with an injector. This protocol can be adapted for use with a luminometer with or without an injector for kinetic measurements.

1. Add 20 µl of sample per well including a positive control (such as recombinant human IFN-β at 1000 IU/ml) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
2. Add 20 µl of double-stranded DNA complexed to a transfection reagent (such as VACV-70/LyoVec™ (1 µg/ml final concentration) in another well.
Note: This ligand will induce Lucia luciferase activity in the parental THP1-Dual™ cells but not in THP1-Dual™ KO-cGAS cells.
3. Add 180 µl of cell suspension (∼100,000 cells) per well of a flat-bottom 96-well plate.
4. Incubate the plate for 18-24 h at 37°C, 5% CO2.
5. Prepare QUANTI-Luc™ following the instructions on the enclosed data sheet.
6. 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.
7. Pipet 10 µl of THP1-Dual™ KO-cGAS cells culture medium per well into a 96-well white (opaque) or black plate, or a luminometer tube.
8. Prime the injector with the QUANTI-Luc™ assay solution and proceed with the measurement.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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<tbody>
<tr>
<td>2′,3′cGAMP</td>
<td>Cyclic [G(2′,5′)pA(3′,5′)p]</td>
<td>thr-lacg23</td>
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<td>Blasticidin</td>
<td>Selective antibiotic</td>
<td>ant-bl-1</td>
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<td>Normocin™</td>
<td>Antimicrobial agent</td>
<td>ant-nr-1</td>
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<td>QUANTI-Blue™</td>
<td>SEAP detection medium</td>
<td>rep-gb1</td>
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<td>QUANTI-Luc™</td>
<td>Secreted luciferase detection medium</td>
<td>rep-qlc1</td>
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<td>Recombinant hTNF-α</td>
<td>Recombinant human cytokine</td>
<td>ryc-hmlfa</td>
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<td>THP1-Dual™</td>
<td>Parental cell line</td>
<td>thpd-nifs</td>
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<td>VACV/LyoVec™</td>
<td>Viral DNA motif &amp; transfection reagent</td>
<td>thr-vav70c</td>
</tr>
<tr>
<td>Zeocin™</td>
<td>Selective antibiotic</td>
<td>ant-zn-1</td>
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</table>

Cell Preparations
Use THP1-Dual™ KO-cGAS cells with their corresponding parental (wild-type) cell line, THP1-Dual™ cells.
1. Centrifuge cells at 1000-1500 RPM (RCF 200-300 g) for 5 minutes.
2. Remove supernatant and resuspend THP1-Dual™ KO-cGAS cells at 5 x 10⁵ cells/ml in fresh, pre-warmed growth medium.

Cell Handling Recommendations
To ensure the best results:
- Use THP1-Dual™ KO-cGAS 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% CO2.
QUANTI-Blue™
Medium for detection and quantification of alkaline phosphatase
Catalog # rep-qb1, rep-qb2
http://www.invivogen.com/quanti-blue

For research use only
Version # 16C18-MM

PRODUCT INFORMATION

Contents:
QUANTI-Blue™ is provided as packs of individually sealed pouches.
• rep-qb1: 5 pouches of QUANTI-Blue™
• rep-qb2: 10 pouches of QUANTI-Blue™
Each pouch contains everything needed to prepare 100 ml of medium for the detection and quantification of any alkaline phosphatase.

Storage and Stability:
- Store QUANTI-Blue™ pouches at 2-8 °C for 12 months.

Important: The correct storage temperature for this product is 2-8 °C (some pouches may be mislabeled).
- Reconstituted QUANTI-Blue™ medium is stable for 2 weeks at 2-8 °C and for 2 months at -20 °C. Keep reconstituted QUANTI-Blue™ away from light.

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™ medium changes 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 that are exploited by the use of QUANTI-Blue™.

- Requires small samples of cell supernatants - Samples of 10 µl are sufficient.
- No need to process samples - Preparation of cell lysates or heating of samples are not required.
- Determine secreted AP activity without disturbing cells - The same cell cultures can be repeatedly sampled for kinetic studies or further experimentation.
- 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™.
- 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 resulting in more significant differences between non or low AP expression and high AP expression.
- Extremely simple to use - QUANTI-Blue™ consists of only one medium: 1) resuspend in water, 2) add sample, incubate at 37 °C and 3) assess AP activity with the naked eye or by reading the optical density (OD) at 625-655 nm.

METHODS

Preparation of QUANTI-Blue™
- Pour the contents of one pouch of QUANTI-Blue™ in a 250 ml sterile glass bottle or flask.
- Add 100 ml of endotoxin-free water.
- Swirl gently.
- Warm QUANTI-Blue™ to 37 °C for 30 min.
- Use reconstituted QUANTI-Blue™ immediately or store at 2-8 °C.

Notes:
- QUANTI-Blue™ may require overnight incubation at 2-8 °C to ensure complete dissolution of the powder.
- Optional: To guarantee sterility, QUANTI-Blue™ can be filtered on a 0.2 µm membrane once complete dissolution is achieved. However, this step is not necessary as your cells will not be in contact with QUANTI-Blue™.

Detection of SEAP activity from cell culture supernatants
The following protocol refers to the use of 96-well plates. Vary your procedure accordingly depending on volumes of reagents needed based on the size of your wells. Some fetal bovine serum (FBS) may contain alkaline phosphatase that can interfere with SEAP quantification. We recommend to test the culture medium supplemented with FBS as a negative control to evaluate the presence of alkaline phosphatase in the serum.
- Aliquot 200 µl QUANTI-Blue™ per well.

Note: Warm QUANTI-Blue™ to 37 °C before use.
- Add 20 µl supernatant of SEAP-expressing cells or cell culture medium as a negative control.

Note: If the negative control turns purple/blue, it means your FBS contains alkaline phosphatase. We recommend to heat the FBS used in your cell culture medium at 56 °C for 30 minutes to inactivate the alkaline phosphatase activity.
- Incubate at 37 °C.
- After 15 min to 24 h incubation, assess SEAP activity with the naked eye or by reading the OD at 620-655 nm with a microplate reader.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
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<tr>
<td>pNiFty2-SEAP (Zeo+)</td>
<td>pnifty2-seap</td>
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<td>pSELECT-zeo-SEAP</td>
<td>psetz-seap</td>
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<tr>
<td>Recombinant SEAP Protein</td>
<td>rec-hseap</td>
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TECHNICAL SUPPORT
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InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com
QUANTI-Luc™
A coelenterazine-based luminescence assay reagent
Catalog # rep-qlc1, rep-qlc2
http://www.invivogen.com/quanti-luc
For research use only
Version # 17C17-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™ 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™ reporter cell lines (see Related Products). Lucia™ is a new secreted coelenterazine luciferase encoded by a synthetic gene. As Lucia™ is secreted, it can be directly measured in the cell culture medium using bioluminescent assays.

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

METHODS

Preparation of QUANTI-Luc™
Prepare the QUANTI-Luc™ assay solution as follows:
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.

Detection of luciferase activity from cell culture medium
Prepare the QUANTI-Luc™ assay solution as described above.

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 the following parameters: 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. 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.

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<tr>
<td>pNiFty3-Lucia™ (reporter plasmid)</td>
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<tr>
<td>pSelect-zeo-Lucia™ (expression plasmid)</td>
<td>psetz-lucia</td>
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<td>RAW-Lucia™ ISG cells</td>
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<td>Recombinant Lucia™ protein</td>
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<tr>
<td>THP1-Lucia™ NF-kB cells</td>
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