RAW-Dual™ Cells  
(IRF-Lucia/KI-[MIP-2]SEAP) 
Dual IRF and MIP-2 (NF-κB) reporter mouse macrophages  
Catalog # rawd-ismip  
http://www.invivogen.com/raw-dual  
For research use only  
Version # 17A05-MM  

PRODUCT INFORMATION  

Contents and Storage  
• 1 vial of RAW-Dual™ (IRF-Lucia/KI-[MIP-2]SEAP) 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 Zeocin™ (100 mg/ml). Store Zeocin™ at 4 °C or at -20 °C.  
• 1 ml Normocin™ (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20°C.*  
• 1 pouch of QUANTI-Blue™ (SEAP detection medium). Store QUANTI-Blue™ pouch at 4°C for 12 months. Reconstituted medium is stable for 2 weeks at 4 °C. Protect QUANTI-Blue™ from light.  
• 1 pouch of QUANTI-Luc™. Store QUANTI-Luc™ pouch at -20 °C for 12 months. Reconstituted QUANTI-Luc™ medium is stable for 1 week at 4 °C and 1 month at -20 °C. Protect QUANTI-Luc™ from light.  
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 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.  

Quality Control  
• The biallelic replacement of the mouse MIP-2 (macrophage inflammatory protein-2; also known as CXCL2) open reading frame (ORF) with the SEAP reporter ORF was verified by PCR and sequencing. Furthermore, the inability to produce MIP-2 has been confirmed by ELISA.  
• Activity of RAW-Dual™ cells was tested with pattern recognition receptor (PRR) ligands that trigger the NF-κB and interferon regulatory factor (IRF) signaling pathways.  
• The stability of this cell line for 20 passages following thawing has been verified.  
• RAW-Dual™ cells are guaranteed mycoplasma-free.  

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 RAW-Dual™ cells more than 20 times. RAW-Dual™ cells should be maintained in growth medium supplemented with Zeocin™ (200 µg/ml) following every other passage.  

PRODUCT DESCRIPTION  
RAW-Dual™ (IRF-Lucia/KI-[MIP-2]SEAP) cells are derived from RAW 264.7 macrophages, a murine immune cell model that expresses many PRRs such as the Toll-like receptors (TLRs) TLR2 and TLR4, and the cyclic dinucleotide sensor STING. RAW-Dual™ cells respond to PRR ligands that trigger the NF-κB and the IRF pathways. They also respond to murine, but not human, IFN-α and IFN-β. These cells stably express two different secreted reporter genes encoding SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase. The Lucia luciferase gene is under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. The MIP-2 ORF has been replaced by the SEAP ORF using knockin technology. Hence, the expression of SEAP depends on the activation of the endogenous MIP-2 promoter. MIP-2, the murine ortholog of IL-8, is a chemokine which is produced in an NF-κB dependent-manner. Both reporter proteins are readily measurable in the cell culture supernatant by using QUANTI-Blue™ and QUANTI-Luc™, SEAP and Lucia luciferase detection reagents respectively. As a result, RAW-Dual™ cells allow to simultaneously study the NF-κB pathway, by assessing the activity of SEAP, and the IRF pathway, by monitoring the activity of Lucia luciferase. RAW-Dual™ Cells are resistant to Zeocin™.  


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.
SAFETY CONSIDERATIONS
Biosafety Level 2

HANDLING PROCEDURES

Required Cell Culture Medium
• Growth Medium: DMEM, 4.5 g/l glucose, 10% fetal bovine serum (FBS), 100 μg/ml Normocin™, 2 mM L-glutamine
• Test Medium: DMEM, 4.5 g/l glucose, 10% heat-inactivated FBS (30 min at 56 °C), 100 μg/ml Normocin™, 2 mM L-glutamine

Note: Some fetal bovine serum (FBS) may contain alkaline phosphatases that can interfere with SEAP quantification. To ensure that these thermosensitive enzymes are inactive, use heat-inactivated FBS. Heat-inactivated FBS is also commercially available.

• Freezing Medium: DMEM, 4.5 g/l glucose, 20% FBS, 10% DMSO

Required Selective Antibiotic
• 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 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 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 prepared extemporaneously with cold growth medium.

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

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. To maintain selection pressure, add 200 μg/ml of Zeocin™ to the growth medium every other passage.

Note: We recommend the use of Pen-Strep (50 U/ml-50 μg/ml) together with Normocin™ to keep the cells free of microbial contaminants.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: We recommend the use of a cell scraper to detach cells.

Cell-Handling Recommendations
To ensure the best results:
• Use RAW-Dual™ cells with less than 20 passages.
• Pass cells either 3 or 4 days prior to the reporter assay.
- Three days prior to the reporter assay, seed cells at a cell density of 2.5x10⁶ per cm², which corresponds to 1.9x10⁶ cells in a T-75 culture flask.
- Four days prior to the reporter assay, seed cells at a cell density of 1.3x10⁶ per cm², which corresponds to 1x10⁶ cells in a T-75 culture flask.

Reporter Assay
RAW-Dual™ cells can detect compounds that activate the NF-κB with the SEAP assay and the IRF pathway using the Lucia luciferase assay.

Cell Preparation
1. Remove medium from RAW-Dual™ cells and rinse twice with pre-warmed PBS.
2. Use a cell scraper to detach cells and resuspend cells in test medium which contains 10% (v/v) heat-inactivated FBS and prepare a cell suspension at ~550,000 cells/ml.

IMPORTANT: To ensure reliable and reproducible results, homogenize the cell suspension.

NF-κB Induction
1. Add 20 μl of sample per well of a flat-bottom 96-well plate, including a positive control (e.g. Pam3CSK4 or LPS-EK Ultrapure at 1 μg/ml final concentration) and endotoxin free water as a negative control.

Note: Use new tips for each well to avoid cross-contamination.
2. Add 180 μl of cell suspension (~100,000 cells) per well.
3. Incubate the plate at 37 °C in a CO2 incubator for 18-24 h.
4. Prepare QUANTI-Blue™ following the instructions on the enclosed data sheet.
5. Add 170 μl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.
6. Add 30 μl of RAW-Dual™ cells supernatant.
7. Incubate the plate at 37 °C in a CO2 incubator for 1-8 h.
8. Determine NF-κB-induced SEAP levels using a microplate spectrophotometer at 620-655 nm.

IRF Induction
Below is a protocol for end-point readings using a luminometer, this protocol can be adapted for use with kinetic measurements.
1. Add 20 μl of sample per well including a positive control (e.g. recombinant mIFN-β at 1x10⁴ U/ml or 2’3’-cGAMP at 10 μg/ml final concentration) and endotoxin free water as a negative control.

Note: 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 at 37 °C in a CO2 incubator for 18-24 h.
4. Prepare QUANTI-Luc™ following the instructions on the enclosed data sheet.
5. Pipet samples (20 μl per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
6. Add 50 μl of QUANTI-Luc™ per well.
7. Proceed immediately with the measurement.

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