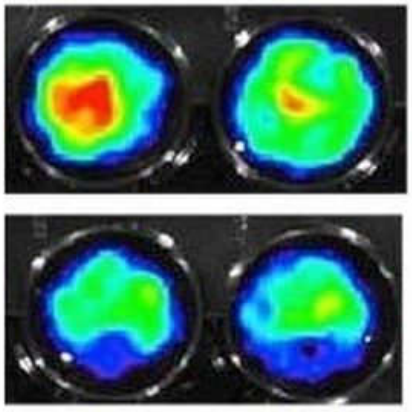
Autobioluminescent Human Liver Cells (HepG2) 1 ml



Autobioluminescent Human Liver Cells (HepG2)

Growth Format: Adherent

Common Applications:

- Cytotoxicity screening
- Metabolic activity monitoring
- New compound development screening

490 BioTech's autobioluminescent HepG2 cell line is an excellent choice for cytotoxicity screening and metabolic activity monitoring. This cell line naturally express a wide range of detoxification enzymes, making them ideal candidates for new compound development screening assays.

Category: Human Cell Lines

Product Sheet

Autobioluminescent HepG2 Cells

Handling Upon Receipt

Inspect packaging for damage or leaks. Remove the vial containing cells from dry ice and store in liquid nitrogen vapor (< -130 °C) or immediately thaw and culture.

Warranty

This product is warranted viable for 30 days from the date of shipment and is valid only if the product is stored and cultured according to the information provided on this sheet. The use of medium components other than those recommended by 490 BioTech on this sheet will invalidate this warranty.

Disclaimers

While 490 BioTech makes every effort to include accurate and up to date information on this product information sheet, 490 BioTech makes no warranties or representations of its accuracy. This product is sent with the condition that you are responsible for its safe storage, handling, and use. 490 BioTech is not liable for any damages or injuries arising from the receipt and/or use of this product.

Limited Use Label License

This product is to be used for internal, non-commercial research purposes for the sole benefit of the purchaser. It may not be used for any other purpose, including, but not limited to diagnostics or therapeutics, and may not be used in humans. This product may not be transferred or sold to third parties, resold, modified for resale, or used to manufacture or develop commercial products or to provide a service of any kind to third parties, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining commercial research or additional rights, please contact 490 BioTech at licensing@490biotech.com.

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Cellular Background

Organism: *Homo sapiens*
Tissue Type: Liver
Growth Format: Adherent

Recommended Growth Medium

The following medium is recommended for cellular growth:

- RPMI-1640
- 10% FBS
- 1X Antibiotic/Antimycotic
- 200 µg G418/ml*
  *It is recommended that G418 be omitted during screening

Thawing Frozen Cells

1. Incubate the vial in a 37 °C water bath with gentle agitation until contents have thawed (thawing should occur in approximately 2 minutes). To reduce the possibility of contamination, do not submerge the O-ring or cap during the thawing process.
2. Spray the thawed vial with 70% ethanol and transfer to a sterilized environment. All remaining steps should be performed using aseptic technique.
3. Transfer the full contents of the vial to a 15 ml centrifuge tube containing 9 ml of complete growth medium pre-incubated to 37 °C and centrifuge at 125 x g for 7 minutes.
4. Resuspend the cell pellet in complete growth medium pre-incubated to 37 °C and transfer to an appropriate cell culture vessel. 25 cm^2 culture flasks are recommended for initial thawing procedures.
5. Incubate the cells at 37 °C 5% CO_2 in a humidity controlled environment and monitor for growth.

Routine Growth and Maintenance

1. Remove and discard spent culture medium.
2. Rinse cells with an appropriate volume of sterile, 37 °C PBS.
3. Add an appropriate volume of Trypsin-EDTA solution to the flask and incubate until cells have detached (detachment usually occurs within 2 to 15 minutes depending on if the incubation is performed at room temperature or at 37 °C).
4. Resuspend the detached cells in an appropriate amount of pre-incubated 37 °C complete growth medium.
5. Aliquot cells into new culture vessels containing pre-incubated 37 °C complete growth medium.
6. Incubate the cells at 37 °C 5% CO_2 in a humidity controlled environment.

Subcultivation ratio:	It is recommended that cells be subcultured at a ratio between 1:3 and 1:6 as needed.
Medium Refreshment:	It is recommended that medium be refreshed every 2 to 3 days as needed.

Biosafety Information

These cells are treated as biosafety level 1. Appropriate safety procedures should always be followed when working with or using these cells. More information can be obtained from the US Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health's *Biosafety in Microbiological and Biomedical Laboratories*.

The substrate free revolution.

rev 100816

FAQ'S

About Us

490 BioTech is a life sciences company that develops patent protected light-emitting (bioluminescent) human cell lines genetically programmed to 'report' on biological events or interactions that affect their metabolic status. The incorporation of these cell lines into cell culture and small animal preclinical efficacy/toxicity testing models is designed to accelerate the pace of new drug discovery and move drugs more rapidly and more effectively towards Phase I human testing with a reduced overall cost.

What is autobioluminescence and how is it different than the traditional bioluminescent signal from firefly luciferase?

The only difference between autobioluminescence and traditional bioluminescence is that autobioluminescence is fully self-generated and self-directed by the cell, removing any error or variation resulting from human interaction. This is made possible by engineering the autobioluminescent cells so that they can generate all of the components required to produce a bioluminescent signal internally and without any investigator interaction. This allows them to produce an autobioluminescent signal continuously, or to modulate that signal in response to changes in their environment or genetic expression patterns. Because the autobioluminescent signal is simply light, it can be assayed using the same equipment that you already use to monitor traditional bioluminescent cells, and can generally be interchanged freely with these cells within experimental designs. In short, autobioluminescence is just a simpler, easier way to perform your existing bioluminescent experiments.

What are the benefits of using an autobioluminescent cell line vs. a substrate-dependent cell line?

Autobioluminescent cells can often be used in place of substrate-dependent cell lines to simplify assay design, reduce costs, and increase the amount of data collected in your assays. The autonomous nature of the autobioluminescent signal allows cells to be assayed repeatedly or continuously without destruction, reducing the number of cells that must be prepared for each experiment and reducing sample to sample variability. Because the autobioluminescent signal is completely self-modulated and does not require chemical or photonic stimulation prior to emission, autobioluminescent cells are also more amenable to automated or high throughput assay designs. From a quality control standpoint, the use of autobioluminescent cells removes concerns relating to substrate quality, uptake rates, or application efficiency, and completely removes any chance for unintended substrate/treatment interactions. In general, if a substrate-dependent cell line is used, an autobioluminescent cell line can be substituted to perform the same assay with less cost and less investigator effort, all while providing you with increased data acquisition through continuous monitoring.

Do autobioluminescent cells require different maintenance protocols than wild type or firefly luciferase-expressing cell lines?

No. Autobioluminescent cells grow and divide just like wild type or firefly luciferase-expressing cell lines and can be maintained using the protocols you already have in place. In fact, because autobioluminescent cell lines do not require destruction or external stimulation to generate their autobioluminescent signal, they are often easier to maintain. Unlike firefly luciferase expressing cells, autobioluminescent cells can be grown, assayed repeatedly or continuously, and then returned to the incubator for continued growth. There is no need to prepare individual samples for each time point. The same cell samples can also be assayed repeatedly to provide technical replicates for each time point, increasing your statistical power.

Can autobioluminescent cells be visualized within small animal models such as mice?

Yes. Autobioluminescent cells can be visualized within small animal models so long as the emission signal is capable of penetrating the tissue between the autobioluminescent cell and the detector. Due to the unique nature of small animal models, it is recommended that preliminary assays be performed in your model system to determine the minimum number of cells and imaging conditions required for reliable detection prior to beginning any new small animal-based research project.

I've only used firefly luciferase or other substrate-requiring reporters before. Will I need any special equipment to assay autobioluminescent cells?

No, your existing equipment is all you need. The autobioluminescent signal is simply light at a wavelength of 490 nm, so plate readers and CCD camera-based equipment that can read other

bioluminescent signals can usually read the 490 nm signal without any modifications. Please consult your equipment specifications for details to ensure that it is capable of capturing a 490 nm emission signal.

I've only used GFP or other fluorescent reporters before. Will I need special equipment to assay autobioluminescent cells?

You may not. The autobioluminescent signal is simply light at a wavelength of 490 nm, so plate readers and CCD camera-based equipment can usually read the 490 nm signal without any modifications. If your equipment can be operated without an excitation signal and with an open emission filter, it will likely be compatible with the autobioluminescent signal without modification. Please consult your equipment specifications for details to ensure that it is capable of capturing a 490 nm emission signal and operating without an excitation signal.

How well does the autobioluminescent signal correlate with alternative assays?

The continuously generated signal from autobioluminescent cells allows them to be used in place of many common, more time consuming, and more expensive assay formats. Strong correlations exist between autobioluminescent dynamics and MTT assays or ATP and reactive oxygen species level-dependent cytotoxicological assays. Similarly, for compound detection assays the signal provides an excellent alternative to destructive E-SCREEN assays. However, to ensure strong correlation under your experimental conditions, it is recommended that side-by-side comparisons be performed and appropriate R^2 values be generated prior to use.

What is the basic procedure for assaying autobioluminescent output?

Autobioluminescent cell lines do not require any investigator intervention prior to signal generation. Therefore, they can be treated as required and assayed repeatedly as needed with no special treatment. For long-term assay designs it is recommended that cells be maintained in a temperature/humidity/CO_2 controlled environment or returned to an incubator between readings, but no special treatment or handling is otherwise required. For most experimental designs the cells are simply processed as needed, placed into the detector, assayed repeatedly, and then discarded or returned for further growth and/or processing.

Is there a preferred culture medium for signal acquisition?

The autobioluminescent signal is strongest when cells are healthy and metabolically active. We therefore recommend that cells be assayed in their preferred culture medium. If possible, phenol red should be omitted from the medium because of its photon absorption properties. However, its effects will be minimal if its use is required and it can be included if necessary.

What is the half-life of the optimized bacterial luciferase?

Because autobioluminescent cells have been engineered to self-produce all of the components required for signal generation, the autobioluminescent reaction occurs continuously when the cassette is expressed constitutively. This means that, unlike substrate-dependent luciferase systems such as firefly luciferase, instead of continuously exhausting a limited supply of externally applied substrate the autobioluminescent cells will continue to self-synthesize both the luciferase enzyme and its required substrates. This results in continuous autobioluminescent output that is maintained as long as the cell is metabolically and transcriptionally/translationally active. Because the optimized bacterial luciferase cassette is genetically encoded, this phenotype is also passed on to each daughter cell during cell division, allowing populations of cells to be tracked over extended time periods with no external interaction required. These unique attributes mean that the half-life of the autobioluminescent signal is functionally unlimited as long as the cellular population remains healthy.

How stable is the autobioluminescent signal following multiple passages?

We have found the autobioluminescent signal to remain stable over multiple passages, however, it is impossible to account for all cellular changes that result from normal growth and maintenance. As such, we recommend maintaining cultures for no more than 10 – 15 passages. This will ensure that the autobioluminescent signal remains stable and will minimize intracellular changes that could affect assay results.

Is it possible to maintain/assay autobioluminescent cells without G418 or other antibiotic markers?

Just as with any transfected cell line, continuous, low level selective pressure will help to ensure the optimized bacterial luciferase gene cassette remains actively expressed from within the cellular genome. While it has been demonstrated that cells often retain their autobioluminescent phenotype even without selective pressure, we do not recommend that antibiotic usage be discontinued during regular growth and maintenance. While doing so can increase cellular growth rates, it can also lead to reductions in autobioluminescent output due to the loss of the cassette from part or all of the population. However, if the potential influence of the antibiotic marker on your specific experimental design is a concern, it can usually be removed during the assay period without significant negative effects.

What is the minimum acquisition time for assaying autobioluminescent output?

The minimum acquisition time required for assaying autobioluminescent output is dependent on a number of factors such as treatment conditions, the number of cells being observed, the sensitivity of the detection equipment being used, and the size of the observed area. We recommend that the acquisition time be empirically determined for each experiment by performing an initial analysis consisting of decreasing acquisition times ranging from 10 minutes to 1 second at each time point you wish to observe.

What is the minimum number of cells required to observe an autobioluminescent signal?

The minimum number of cells required to observe an autobioluminescent signal is dependent on a number of factors such as your treatment conditions, the sensitivity of the detection equipment, and size of the observed area. We recommend that the minimum cell number be empirically determined for each experiment by performing an initial analysis using decreasing cell populations. We recommend that 5×10^4 cells/well in a 96 well format be used as a starting point for determining experimentally relevant cell population sizes.

Why does autobioluminescent output seem to increase the longer I leave my cells at room temperature?

Autobioluminescent output often increases during incubation at room temperature under atmospheric conditions. This is due to changes in the cellular microenvironment that enhance the autobioluminescent reaction. Unfortunately, these same conditions also promote signal output variability. It is recommended that cells not be incubated under atmospheric conditions longer than 15 minutes prior to acquisition, as longer incubations begin to promote increases in signal variability that could deleteriously affect results.

My signal-to-noise ratio is low. Is there a way that I can increase autobioluminescent output so I can improve signal detection?

There are several ways to increase the signal-to-noise ratio of autobioluminescent cells. The most effective method is to reduce the surface area or volume in which the cells are housed (i.e., perform acquisition in a 96 well plate rather than a 24 well plate). If this is not feasible, the number of cells assayed can be increased, opaque plates can be used in place of transparent plates, or a short (< 15 minutes) room temperature pre-incubation can be employed to increase autobioluminescent output.

I am having trouble transfecting the optimized gene cassette into my cell line. How can I improve the efficiency of this process?

The optimized autobioluminescent cassette is much larger than a single gene construct such as firefly luciferase, and therefore is often more difficult to transfect. We recommend an electroporation-based transfection protocol due to the large size of the cassette, but other methods have been shown to work as well. In general, the transfection process is detrimental to autobioluminescent output kinetics, and we therefore do not recommend screening at the individual colony level. Following transfection and selection, isolated colonies should be passaged in tandem into paired wells of 24 well and 6 well plates. Upon reaching 85% confluence, each well of the 6 well plate, representing each isolated colony, should be harvested and resuspended in 200 µl in a single well of a 96 well plate. This plate can then be assayed to assess the autobioluminescent output of all clonal lineages simultaneously and the wells from the 24 well plates representing those lineages with the greatest level of

autobioluminescent output can be scaled up for further evaluation. If transient transfection is being performed, each well of the 6 well plate used for transfection should be pooled, resuspended in 200 µl in a single well of a 96 well plate, and used directly for experimental analysis.

I don't see my desired cell type on the website. Is there a way to get an autobioluminescent version of my cells?

We are constantly engineering new autobioluminescent cell lines, so please check back soon. You can also contact us at info@490BioTech.com to discuss contracting development of your cell line, or purchase one of our autobioluminescent DNA vectors to develop your own lines in house.

I like the idea of your technology, but my work does not require human cell screening. What are the limits of 490 BioTech's technology outside of the screening process?

The short answer is, none. 490 BioTech's technology is revolutionary in its ability to continuously and autonomously produce light from human and animal cells. This means that no one yet knows the creative ways it will be used in the future. From personalized medicine that allows doctors to determine how a patients' cells will respond to potential treatments in real time, to glowing fish that can light up both aquariums and children's imaginations, 490 BioTech is breaking the barriers of what is considered possible and is ready to help you bring your next big idea to life.