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Mitochondrial Assay Compass for Energy Metabolism Studies

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Introduction to Energy Metabolism

1.1 Energy Metabolism Pathways

Energy metabolism constitutes the fundamental basis of all biological activities. Following the uptake of small molecular substrates such as glucose, fatty acids, and amino acids, cells convert the chemical energy stored within these molecules into adenosine triphosphate (ATP), the universal cellular energy currency, through a highly coordinated biochemical process. This process occurs predominantly within mitochondria, which function as highly efficient bioenergetic powerhouses. The principal metabolic pathways involved are Glycolysis, Fatty Acid Oxidation, Tricarboxylic Acid Cycle, Electron Transport Chain, Oxidative Phosphorylation and Amino Acid Metabolism.

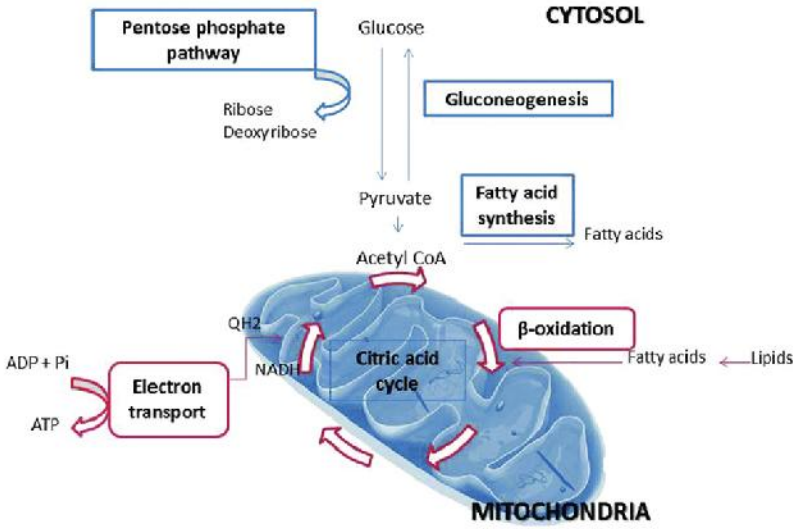


Figure 1: Mitochondria-Driven Energy Metabolic Pathways

Glycolysis

During glycolysis, glucose undergoes initial catabolism within the cytoplasm to generate pyruvate, accompanied by the production of limited amounts of ATP and electron carrier nicotinamide adenine dinucleotide (NADH). Under anaerobic conditions, pyruvate is converted into lactate, through a reaction catalyzed by lactate dehydrogenase (LDH). Lactate is subsequently exported from the cell, via monocarboxylate transporter 4 (MCT4).

Fatty Acid Oxidation

Fatty acid oxidation primarily occurs through β oxidation within mitochondria. In this process, fatty acids are sequentially degraded by multiple enzymatic reactions to generate acetyl coenzyme A (Acetyl CoA). The resulting Acetyl CoA subsequently enters the tricarboxylic acid (TCA) cycle to support cellular energy production.

Tricarboxylic Acid Cycle

Acetyl CoA, derived from pyruvate and fatty acid catabolism, enters the tricarboxylic acid (TCA) cycle within the mitochondrial matrix, where it undergoes complete oxidative metabolism. This process yields high levels of reducing equivalents, including NADH and flavin adenine dinucleotide (FADH2).

Electron Transport Chain and Oxidative Phosphorylation

The electron transport chain and oxidative phosphorylation represent the central ATP generating machinery of cellular metabolism. NADH and FADH2 donate electrons to the electron transport chain located on the inner mitochondrial membrane. The energy released during electron transfer drives the establishment of a transmembrane proton gradient, generating a powerful proton motive force. Protons subsequently flow back along this electrochemical gradient through ATP synthase, thereby driving large scale ATP production. This process is highly dependent on oxygen availability.

Amino Acid Metabolism

In addition to their role in protein biosynthesis, amino acids can undergo metabolic conversion through processes such as deamination to generate TCA cycle intermediates or other bioactive molecules involved in energy production. Representative examples include glutamine and branched chain amino acids.

1.2 Biomarkers of Energy Metabolism and Their Applications

Energy metabolism is intrinsically linked to normal physiological activities and cellular homeostasis. Assessment of metabolic status serves as a critical indicator of cellular health, providing valuable insights into processes such as cell proliferation, differentiation, and apoptosis, while advancing the understanding of disease mechanisms and therapeutic development.

Key biomarkers of energy metabolism encompass metabolites and metabolic enzymes associated with the five principal metabolic pathways. Owing to the broad range of available biomarkers, appropriate indicators may be selected according to specific research objectives and experimental applications. Among the most widely utilized parameters are extracellular acidification rate (ECAR), fatty acid oxidation, oxygen consumption rate (OCR), and ATP production.

Elabscience offers a comprehensive portfolio of energy metabolism assay kits with extensive biomarker coverage, enabling diverse applications across multiple research fields and experimental requirements.

Metabolic Pathways	Number of Detectable Biomarkers	Key Detection Biomarkers	Major Research Applications
Glycolysis	10-20	ECAR, Lactate, Glucose Uptake	✧ Tumor biology, particularly the Warburg effect ✧ Immunometabolism, including T cell and macrophage activation
Fatty Acid Oxidation	10-20	Fatty Acid Oxidation, β-Hydroxybutyrate, Free Fatty Acids	✧ Immunotherapy research ✧ Metabolic disease studies
Amino Acid Metabolism	20-40	Glutamine, Branched-Chain Amino Acids	✧ Tumor microenvironment and immunosuppression research ✧ Cell proliferation and cancer biology
Tricarboxylic Acid Cycle	15-25	Citrate, α-Ketoglutarate, Succinate	✧ Metabolic reprogramming research in cancer and immunology ✧ Epigenetic regulation studies
Oxidative Phosphorylation	8-10	OCR, Mitochondrial Complexes, ATP	✧ Fundamental mitochondrial biology, mitochondrial disorders, and mitochondrial dysfunction research ✧ Neurodegenerative disease research ✧ Drug induced hepatotoxicity and cardiotoxicity assessment

Mapping Energy Metabolism Biomarkers

2.1 Glucose Uptake: An Early Rate Determinant of Cellular Energy Metabolism

Biomarker	Catalog Number	Detection Instrument	Features	Biological Significance
2-Deoxyglucose	E-BC-F041	Fluorescence microplate reader	➢ Non-radioactive assay system ➢ High sensitivity ➢ Suitable for high throughput analysis	Directly reflects the efficiency of glucose uptake mediated by glucose transporters, serving as a dynamic indicator for evaluating cellular metabolic activity and nutrient sensing capacity.
2-NBDG	E-CK-A441	Fluorescence Microscope; Flow Cytometer	➢ Real-time monitoring of live cells ➢ Simple experimental workflow	Enables visualization and quantitative assessment of glucose uptake dynamics in living cells, providing an effective tool for monitoring metabolic activity and glucose utilization.

■ Detection Principle

Glucose analogs share structural similarity with glucose and can be transported into cells via glucose transporters. However, they cannot undergo complete metabolic processing. Therefore, the intracellular accumulation of glucose analogs is quantified to evaluate the glucose uptake capacity of cells.

■ Product Performance

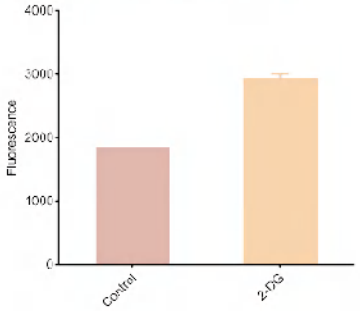


Figure 2. Jurkat cells were incubated with 10 µL of 10 mM 2 deoxyglucose at 37°C for 30 min. Subsequently, glucose uptake was quantified using the Glucose Uptake Fluorescence Assay Kit (E-BC-F041).

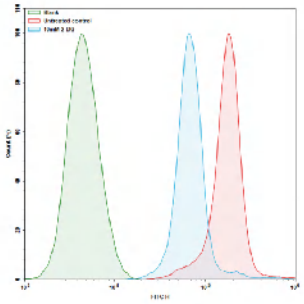


Figure 3. Jurkat cells were cultured in parallel in medium supplemented with or without 2 deoxyglucose (2-DG) and incubated for 60 min. Glucose uptake was then assessed using the 2-NBDG Glucose Uptake Assay Kit (E-CK-A441).

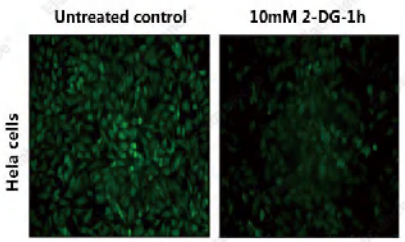


Figure 4. HeLa cells were incubated in the presence or absence of 10 mM 2 deoxyglucose (2-DG) for 60 min. Glucose uptake was subsequently measured using the 2-NBDG Glucose Uptake Assay Kit (E-CK-A441).

■ Literature-Based Case Study

Glucose uptake in Kdm2a WT vs. KO C2C12 cells (cold stimulation)

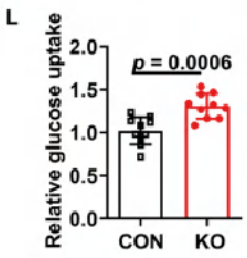


Figure 5. Kdm2a knockout (KO) in C2C12 muscle cells leads to a marked increase in glucose uptake compared to wild-type controls (CON) under cold stimulation (p = 0.0006).

Reference

Kdm2a inhibition in skeletal muscle improves metabolic flexibility in obesity, *Nature Metabolism*, 2025.
DOI: 10.1038/s42255-024-01210-9

2.2 OCR and ECAR: Gold Standard Parameters for Energy Metabolism Assessment

Biomarker	Catalog Number	Functional Significance
Extracellular Acidification Rate (ECAR)	E-BC-F069	Reflects glycolytic activity and serves as an indicator of cellular anaerobic respiration capacity
Oxygen Consumption Rate (OCR)	E-BC-F070	Directly reflects the activity of the electron transport chain (ETC) and ATP synthesis rate, thereby evaluating the capacity of cellular aerobic respiration

■ Detection Principle

ECAR

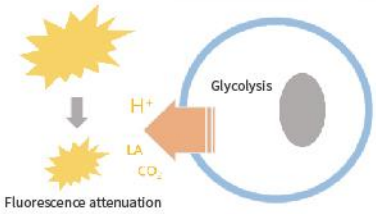


Figure 6. Following the addition of a fluorescent probe, glycolytic activity leads to lactate efflux from cells, resulting in a decrease in extracellular pH. This acidification is accompanied by a corresponding increase in fluorescence intensity of the probe, enabling indirect quantification of glycolytic flux.

OCR

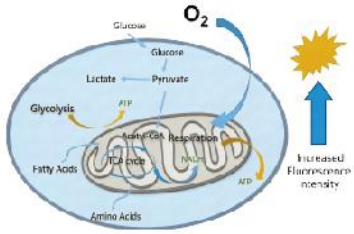


Figure 7. Under sealed or closed measurement conditions, cellular oxygen consumption reduces dissolved oxygen levels. The fluorescent probe exhibits an inverse relationship with oxygen concentration, whereby fluorescence intensity increases as oxygen levels decline. Changes in fluorescence signals are therefore used to determine the oxygen consumption rate of cells.

■ Experimental Procedure

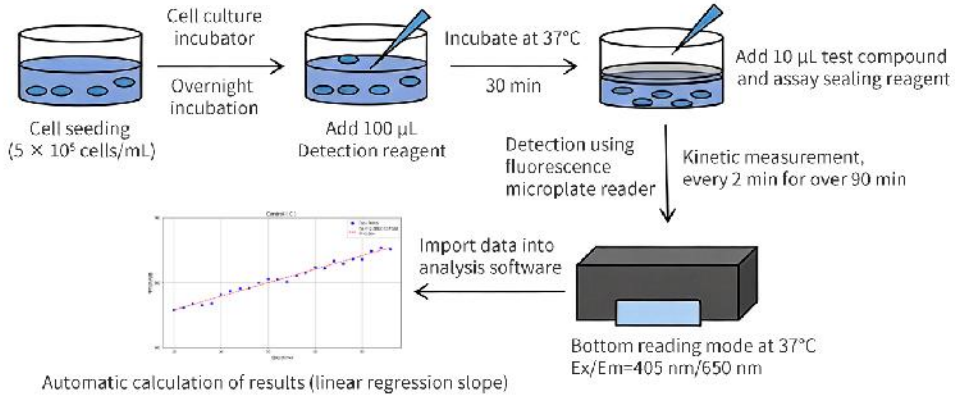


Figure 8. Operational Workflow of OCR

Note: The detection workflows for both OCR & ECAR are similar

Table 1. Comparative Analysis of OCR and ECAR Detection Using Seahorse and Fluorescence Microplate Reader Platforms

Dimension	Seahorse Metabolic Analyzer	Fluorescence Microplate Reader Based Detection
Core Principle	Uses a patented solid-state fluorescent sensor cartridge. The sensor plate is lowered above the cells to form a transient microchamber, enabling non-invasive and real-time simultaneous measurement of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR).	Liquid-phase fluorescent probes are directly added into the wells of a cell culture plate. Oxygen and pH-related changes are detected using a microplate reader.
Instrument Platform	Integrated metabolic analysis system	Multi-functional microplate reader (requires temperature control capability), a general laboratory instrument

Detection Workflow	Standardized workflow with longer preparation time: A. Sensor cartridge hydration and calibration, instrument pre-equilibration for 12 to 24 h B. Cell seeding; suspension cells require immobilization C. Replacement with assay-specific working medium; pre-loading of compounds into sensor cartridge D. Automated real-time detection with sequential compound injection during run	Simplified workflow with shorter preparation time: A. Cells and probe working solution are prepared and added into culture plates B. Instrument preheating for 1 to 2 h C. Initiation of measurement
Compound Injection Capability	Each sample well integrates four automated injection ports, enabling sequential injection of up to four compounds during the assay for real-time kinetic analysis within the same well	No in-run automated compound injection. Experimental groups are typically set up separately to evaluate compound effects
Sample Compatibility	Compatible with both adherent and suspension cells	Compatible with both adherent and suspension cells
Cost and Flexibility	High initial instrument investment and dedicated consumables. Sensor cartridges are single use	Lower overall cost. Probe working solutions can be reused within validity period. More convenient and economical for preliminary experiments. Some kits include pre-formulated compounds, while user-defined reagents are also supported, offering higher flexibility

Product Performance

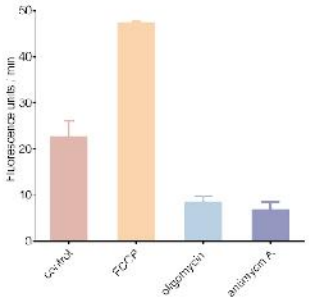


Figure 9. A549 cells were treated with 2 μM FCCP, 1 μM oligomycin, and 1 μM antimycin A, respectively, followed by assessment of changes in oxygen consumption rate (OCR).

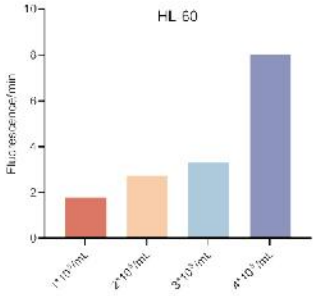


Figure 10. OCR was measured in HL-60 cells at different cell densities. The results demonstrated a clear and proportional increase in signal intensity across all tested densities.

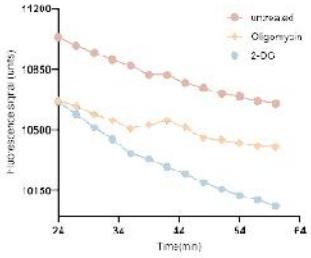


Figure 11. ECAR kinetic profiles of 293T cells were assessed following treatment with 5 μM oligomycin and 0.5 μM 2-deoxyglucose (2-DG), respectively.

Literature-Based Case Studies

Effect of DHX30 Overexpression on Glycolysis in MCF-7 Cells

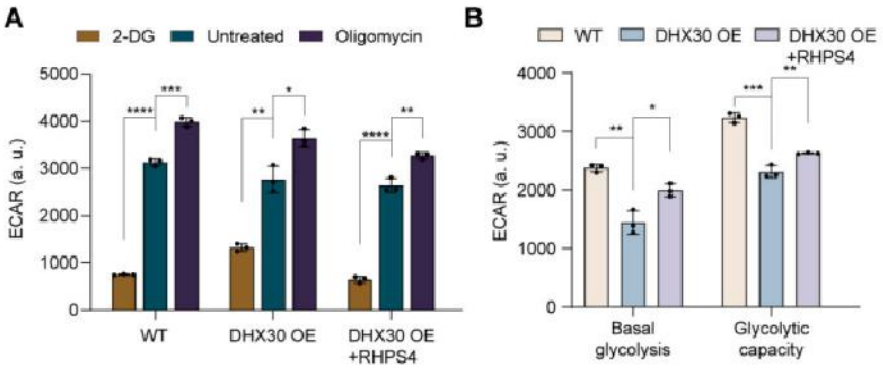


Figure 12. MCF-7 cells with DHX30 overexpression (DHX30 OE) were seeded at a density of 2 × 10^4 cells per well in 96- well plates. After equilibration at 37°C under CO_2 free conditions for 2 h, 100 μL of working solution was added. Extracellular acidification rate (ECAR) was then measured at 490 nm excitation and 535 nm emission wavelengths, with kinetic acquisition performed every 2 min for a total duration of 3 h.

Reference

A subcellular selective APEX2-based proximity labeling used for identifying mitochondrial G-quadruplex DNA binding proteins. *Nucleic Acids Research, 2025.*
DOI: 10.1093/nar/gkae1259

■ OCR & ECAR FAQ's

Q1: Is the recommended seeding density of 50,000 cells per well fixed, or can it be adjusted as long as the same cell number is used within each group?

A: The recommended seeding density provided in the instruction manual is approximately 5×10^4 cells per well, and should be used as a reference only. Due to variations among different cell types and experimental conditions, the seeding density may be adjusted accordingly. In principle, the number of cells seeded per well should remain consistent across all wells. It is recommended that, after overnight culture, the cell density reaches approximately 80% to 90% confluency for optimal results.

Q2: What are the requirements for the fluorescence microplate reader used with the OCR assay kit?

A: The microplate reader must be capable of measuring fluorescence intensity, temperature control, and kinetic measurements, and must include a bottom reading module. The instrument settings for E-BC-F070 are as follows: kinetic mode, bottom reading, 37°C, excitation wavelength 405 nm, emission wavelength 675 nm. Measurements are taken every 2 min for a continuous duration of at least 90 min.

Q3: If cells are transfected with a virus carrying a green fluorescent label (GFP), will the intrinsic green fluorescence affect subsequent ECAR measurement data analysis?

A: Since GFP fluorescence has an excitation wavelength of 488 nm and an emission wavelength of 507 nm, which is close to the detection wavelengths specified in the protocol (excitation 490 nm, emission 535 nm), it may potentially interfere with the measurement results. However, the extent of this influence is not clearly defined. It is recommended to include a blank control well containing only transfected cells and 200 µL of Reagent 1 for kinetic measurement. If the fluorescence signal is high and shows fluctuations over time, the sample may not be suitable for this assay.

Q4: Does the ECAR fluorescence assay kit (E-BC-F069) require construction of a standard curve?

A: This kit is a qualitative assay for ECAR. It evaluates ECAR levels by comparing the rate of linear decrease in fluorescence intensity over time rather than performing quantitative measurement. Therefore, a standard curve is not required.

2.3 Fatty Acid Oxidation: The Third Core Dimension for Precise Interpretation of Energy Metabolism

Biomarker	Catalog Number	Function
Fatty acid oxidation	E-BC-K784-M	Reflects the cellular capacity for lipid catabolism and energy production, serving as a direct indicator for evaluating systemic metabolic flexibility, energy homeostasis, and physiological health status

■ Detection Principle

FAO capacity is assessed by measuring enzyme activity associated with fatty acid oxidation. The principle is illustrated in the figure below. The optical density (OD) of the reaction system is measured at 450 nm using a microplate reader, and the magnitude of the OD value reflects the FAO capacity of the sample.

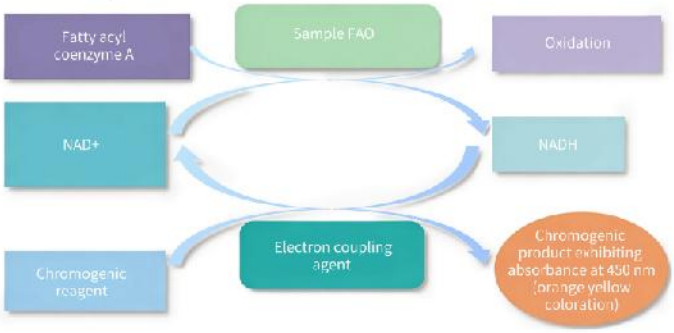


Figure 13. Fatty acid oxidation assay reflecting FAO activity.

■ Experimental Procedure

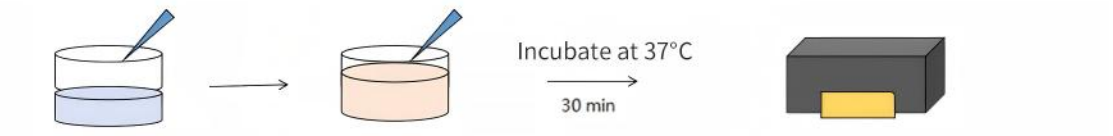


Figure 14. Operational Workflow of FAO

■ Product Performance

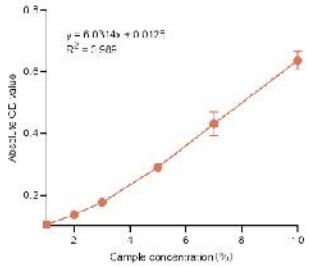


Figure 15. Dilution curve of 10% mouse liver homogenate samples.

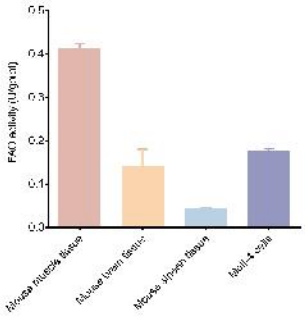


Figure 16. Different sample types measured using the Fatty Acid Oxidation (FAO) colorimetric assay (E-BC-K784-M).

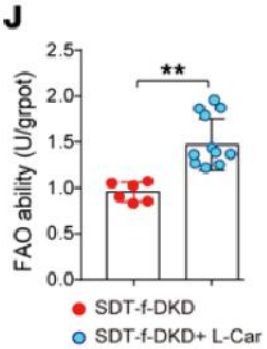
■ Literature-Based Case Studies

FAO activity in kidney tissues across different rat treatment groups.

Figure 17. Kidney samples from SDT-f-DKD rats in the treatment and control groups were homogenized and centrifuged, and the supernatants were collected. Protein concentrations were normalized using the BCA method prior to FAO activity measurement.

Reference

Involvement of impaired carnitine-induced fatty acid oxidation in experimental and human diabetic kidney disease, *JCI Insight*, 2025.
DOI: 10.1172/jci.insight.179362



2.4 ATP/ADP: The Core of Cellular Energy

Biomarker	Catalog Number	Function
ATP/ADP	E-BC-F004	The ATP/ADP balance represents a fundamental determinant of cellular health, and its dynamic changes reflect the final output of cellular energy metabolism and overall physiological status
ATP	E-BC-F300	ATP serves as the direct energy currency of the cell, and its content directly reflects the immediate energy supply capacity

■ Product Performance

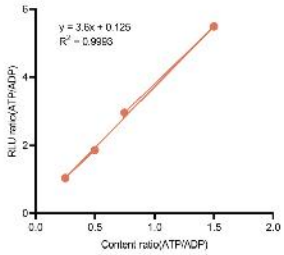


Figure 18. ATP/ADP ratios determined in mixed ATP and ADP samples at different concentrations using the assay kit.

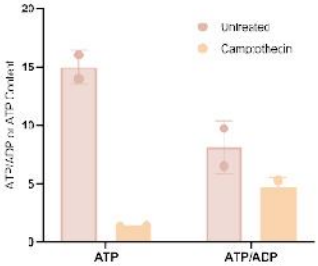


Figure 19. Intracellular ATP levels and ATP/ADP ratio following camptothecin-induced apoptosis were quantified using the ATP/ADP ratio Chemiluminescence Assay Kit (E-BC-F004).

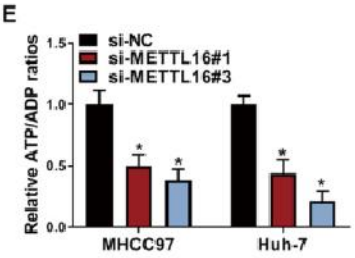
■ Literature-Based Case Study

ATP/ADP results of MHCC97 and Huh-7 cells under different treatments.

Figure 20. MHCC97 and Huh-7 cells were transfected with different siRNAs and collected after 48 h, followed by measurement of intracellular ATP/ADP levels.

Reference.

The promoting effect of the POU3F2/METTL16/PFKM cascade on glycolysis and tumorigenesis of hepatocellular carcinoma, *Annals of Hepatology*, 2025.
DOI: 10.1016/j.aohep.2025.101776



Energy Metabolism Analysis Workflow

3.1 Energy metabolism detection strategy for macrophages

Different functional states of macrophages are driven by distinct energy metabolic programs. Metabolic reprogramming is not only a consequence of functional polarization but also a key regulatory switch controlling immune function. Energy metabolism analysis is primarily applied in the following research areas:

❶ Polarization State Identification

Metabolic parameters such as OCR and ECAR can serve as quantitative and functional indicators for M1 and M2 macrophage classification.

❷ Functional Regulation Studies

Investigations into how signaling pathways such as mTOR and AMPK, as well as metabolites, regulate immune function through metabolic modulation.

❸ Disease Model Studies

In disease models including cancer, atherosclerosis, and obesity, metabolic profiling is used to elucidate how macrophage metabolism influences disease progression.

Table 2. Common Energy Metabolism Biomarkers in Macrophage Research

Metabolic Pathway	Key Biomarker	M1 Type (Pro inflammatory) Characteristics	M2 Type (Anti-inflammatory) Characteristics
Glycolysis	ECAR	Enhanced ECAR, reliance on the Warburg effect for rapid ATP production and biosynthetic precursor generation	Glycolytic activity remains close to basal or resting levels
Mitochondrial respiration	OCR	Reduced oxidative phosphorylation efficiency	High oxidative phosphorylation activity with robust ATP production
Fatty acid metabolism	Fatty acid oxidation	Decreased fatty acid oxidation with increased lipid synthesis to support membrane formation and signaling molecule production	Increased fatty acid oxidation supporting cellular functional maintenance
Amino acid metabolism	Glutamine	Enhanced glutamine catabolism	Reduced glutamine catabolism
TCA cycle	Succinate	Accumulation of succinate	Normal TCA cycle activity with no significant change in succinate levels

■ Experimental Workflow for Macrophage Energy Metabolism Analysis

1.Reagent preparation: BMDM specific culture medium composed of RPMI 1640 basal medium supplemented with 10% FBS, 1% penicillin streptomycin, and 1% L alanyl L glutamine (2 mM). Store at 4°C and use within 1 month.

2.Cell isolation and pretreatment: Bone marrow cells were harvested from the femur and tibia of C57 mice by flushing. Cells were filtered through a cell strainer and collected. Red blood cells were removed using ACK lysis buffer for 30 s, followed by PBS washing and resuspension for cell counting.

3.Primary culture and polarization induction: Cells were seeded at 2×10^5 cells/mL and cultured with M-CSF (20 ng/mL) for 72 h to generate naïve macrophages.

M1 polarization: Medium was replaced and cells were stimulated with LPS (300 ng/mL) and IFN γ (200 ng/mL) for 24 h.

M2 polarization: Medium was replaced and cells were stimulated with IL 4 (20 ng/mL) and IL 13 (20 ng/mL) for 24 h.

4.Cell collection: Culture medium was discarded, cells were washed with PBS, detached using trypsin digestion, resuspended, and counted for downstream experiments.

5.Phenotypic characterization (flow cytometry): 2–5 × 10^5 cells were collected and blocked for Fc receptors using anti CD16/32 antibody. Cells were then incubated with corresponding flow cytometry antibodies at 4°C in the dark for 30 min, followed by washing and analysis.

6.Energy metabolism analysis: Cells were collected and OCR and ECAR were measured according to kit instructions to evaluate metabolic status.

Energy Metabolism Results in Macrophages

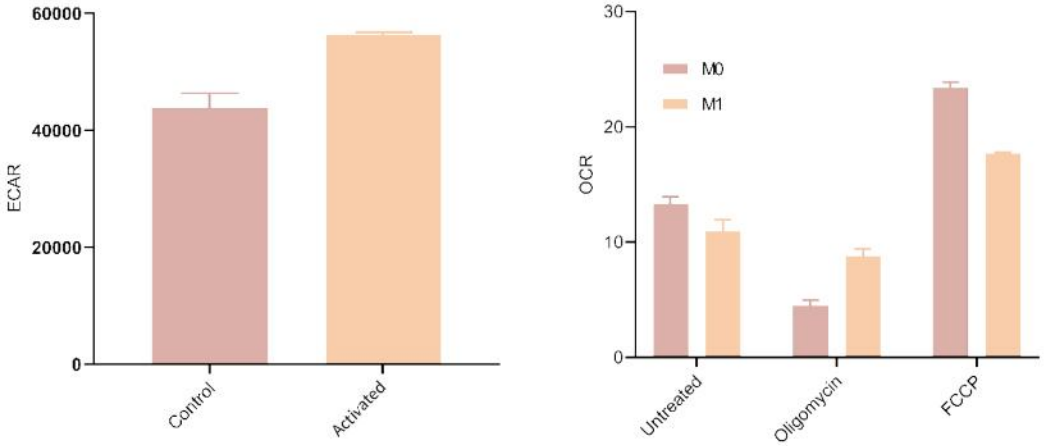


Figure 21. RAW264.7 macrophages were induced into M1 polarization and analyzed using the E-BC-F069 and E-BC-F070 assay kits to measure ECAR and OCR, respectively. The results showed an increase in ECAR, indicating enhanced glycolytic flux, while OCR was reduced, reflecting suppressed oxidative phosphorylation activity. These findings indicate that M1 macrophage polarization is characterized by a metabolic shift toward increased glycolysis.

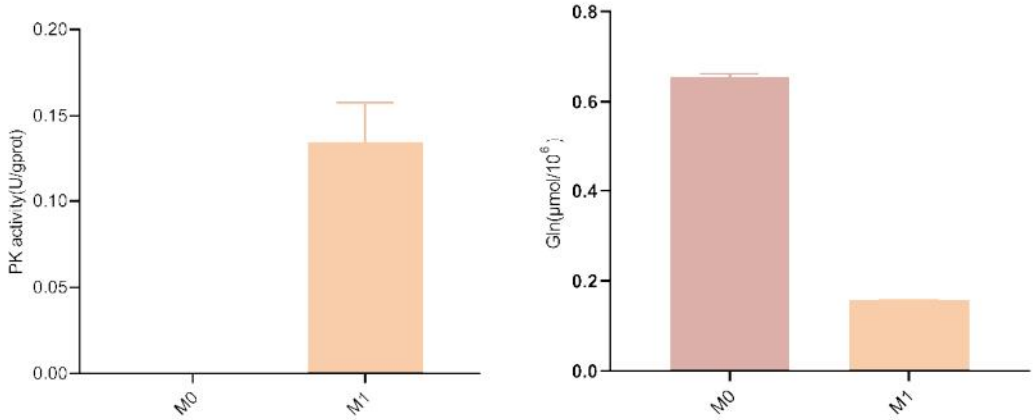


Figure 22. Following induction of M1 polarization in RAW264.7 macrophages, pyruvate kinase activity (E-BC-K611-M) and intracellular glutamine content (E-BC-K853-M) were measured. The results demonstrated that M1 polarization significantly increased pyruvate kinase activity while reducing intracellular glutamine levels.

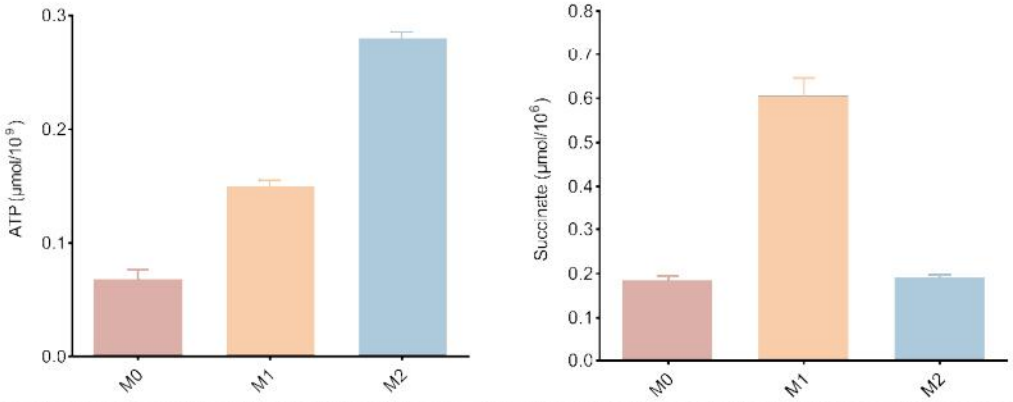


Figure 23. BMDM macrophages were differentiated into M1 and M2 phenotypes, followed by measurement of intracellular ATP (E-BC-F002) and succinate (E-BC-K902-M) levels. The results showed an overall increase in cellular ATP content after polarization. Notably, succinate levels were significantly elevated in M1 macrophages, whereas no obvious change was observed in M2 macrophages.

■ Key Considerations

⚠ **Cell seeding density**

Cell density is critical for experimental success. Excessively high density may lead to hypoxia or extracellular acidification, thereby affecting data quality. A preliminary titration experiment is recommended for optimization.

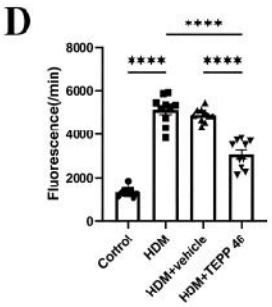
⚠ **Data normalization**

At the end of the assay, cell numbers should be quantified using a nuclear staining dye. Metabolic rates should be normalized to cell number to ensure data accuracy and comparability.

⚠ **Polarization validation**

Prior to and following metabolic analysis, macrophage polarization must be confirmed by detecting marker genes using qPCR or flow cytometry. Typical markers include M1 (iNOS, TNF-α) and M2 (Arg1, CD206).

■ Literature-Based Case Study



Extracellular acidification rate (ECAR) analysis in different groups of BMDMs.

Figure 24. BMDMs were seeded at 2 × 10^5 cells/mL in a 96 well black plate (100 µL per well). The extracellular acidification rate of the untreated group and the HDM (20 ng/mL) treated group was evaluated using the Elabscience E-BC-F069 assay kit.

Reference

Dysregulation of PKM2 promotes inflammatory response in allergic rhinitis, *Inflammation Research*, 2025.

DOI: 10.1007/s00011-025-02097-2

3.2 Energy metabolism analysis strategy for T cells

T cells exhibit distinct metabolic profiles across different functional states, including naïve, activated, effector, and memory stages. Metabolic reprogramming serves as a central regulatory mechanism governing T cell fate determination and functional execution. Energy metabolism analysis is applicable to thefollowing research areas:

❶ Immunotherapy Optimization

Evaluation of the metabolic fitness of CAR T and TCR T cell products, and optimization of in vitro culture conditions.

❷ T-cell Exhaustion Studies

Investigation of the relationship between metabolic impairment, such as mitochondrial dysfunction, and T cell functional exhaustion.

❸ Vaccine Development

Exploration of how vaccine adjuvants modulate metabolic pathways to promote the formation of memory T cells.

Table 3. Metabolic Characteristics and Functional Significance of Different T Cell Subsets

T Cell Subset	Major Metabolic Features	Key Detection Indicators	Functional Significance
Naïve T cells	Predominantly oxidative phosphorylation with low energy demand	Low and stable basal OCR	Maintains cell survival and quiescent state
Effector T cells	Enhanced glycolysis with high energy demand	Markedly increased ECAR and elevated ATP levels	Provides biosynthetic support for rapid proliferation and cytokine production
Memory T cells	Enhanced fatty acid oxidation	High FAO activity and increased OCR spare respiratory capacity	Sustains long term survival and high metabolic flexibility
Regulatory T cells	Increased fatty acid oxidation	Relatively elevated FAO flux	Supports suppressive function and stability in inflammatory environments

T cell Activation Energy Metabolism Detection Workflow

① Single cell suspension preparation:

Mice in the control and disease groups were euthanized by cervical dislocation, and spleens were collected. Spleen tissue was mechanically dissociated through a 70 μm cell strainer and rinsed with pre-cooled PBS. The cell suspension was collected into a 15 mL centrifuge tube and centrifuged at 300 g for 5 min. After centrifugation, the supernatant was discarded.

Each spleen was resuspended in 2 mL freshly prepared 1× ACK lysis buffer and gently pipetted to mix, followed by incubation at room temperature for 2 min for red blood cell lysis.

The lysis reaction was terminated by adding 10 mL PBS, followed by centrifugation at 300 × g for 5 min.

After centrifugation, the supernatant was removed and cells were resuspended in 1 mL culture medium. The suspension was filtered through a 70 μm cell strainer into a 2 mL EP tube, and the final cell concentration was adjusted to 1 × 10^8 cells/mL.

② T cell Activation and Energy Metabolism Analysis:

CD4+ T cells were isolated using a mouse CD4+ T cell negative selection kit (MIM002N). The purified CD4+ T cells were stimulated using CD3/CD28 activation magnetic beads (MIM001A) and cultured at 37°C for 3 days. Subsequently, extracellular acidification rate (ECAR), oxygen consumption rate (OCR), and intracellular ATP levels were measured.

Energy Metabolism Analysis Results of T Cell Activation

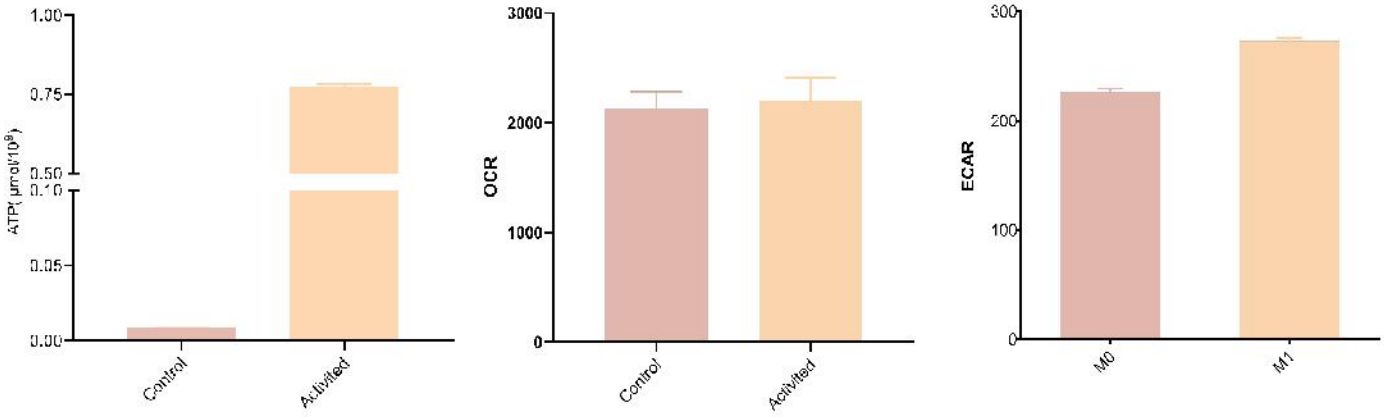
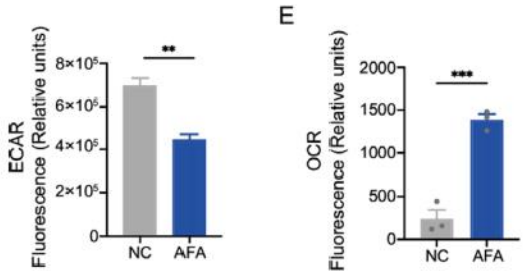


Figure 25. Naïve mouse CD8+ T cells were activated using Mouse CD3/CD28 T Cell Activation Beads (MIM001A) for 72 h, followed by assessment of T cell metabolic status.

Literature-Based Case Study

Changes in OCR and ECAR in T cells treated with AFA.

Figure 26. CAR T cells were treated with AFA for 2 days and resuspended in RPMI 1640 medium containing ECAR and OCR assay working solutions at a cell density of 5 × 10^6 cells/mL. Cells were seeded into black wall clear bottom plates and incubated at 37°C for 30 min. Data were subsequently acquired using a SpectraMax iD5 fluorescence microplate reader.



Reference

Afatinib boosts CAR-T cell antitumor therapeutic efficacy via metabolism and fate reprogramming, *Journal for ImmunoTherapy of Cancer*, 2024. DOI: 10.1136/jitc-2024-009949

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Selected High Impact Citations

Research Area	Detection Marker	Literature	IF
Metabolic diseases	L-Lactic Acid	Meng, Jian-Jun, et al. Light modulates glucose metabolism by a retina-hypothalamus-brown adipose tissue axis. *Cell*, 2023, Jan 19;186(2):398-412.	66.8
	Triglyceride	Wang H, Wang J, Cui H, et al. Inhibition of fatty acid uptake by TGR5 prevents diabetic cardiomyopathy. *Nature Metabolism*, 2024, 6(6):1161-1177.	20.8
Immunotherapy	LDH	Polcaro G, Liguori L, Manzo V, et al. rs822336 binding to C/EBPβ and NFIC modulates induction of PD-L1 expression and predicts anti-PD-1/PD-L1 therapy in advanced NSCLC. *Molecular Cancer*, 2024, Mar 25;23(1):63.	37.3
	ROS	Bai X, Meng F, Wang X, et al. Photodynamic gel-bombs enhance tumor penetration and downstream synergistic therapies. *Signal Transduction and Targeted Therapy*, 2025, 10(1):94.	40.8
Macrophages	α-KGDH	Xia W, Mao Y, Xia Z, Cheng J, Jiang P. Metabolic remodelling produces fumarate via the aspartate-argininosuccinate shunt in macrophages as an antiviral defence. *Nature Microbiology*, 2025, 10(5):1115-1129.	20.5
	ROS	Li L, Jiao Q, Yang Q, et al. A bladder-blood immune barrier constituted by suburothelial perivascular macrophages restrains uropathogen dissemination. *Immunity*, 2025, 58(3):568-584.	25.5
Neurological diseases	OCR	X. Cao, K. Shuai, P. Wang, et al. Biosponge-Armored Nanodots Restore Redox-Calcium Homeostasis to Mitigate Reperfusion-Induced Injury in Ischemic Stroke. *Advanced Functional Materials*, 2025, 35(50).	19.0
Ferroptosis	FFA	Gao Y, Song Z, Gan W, et al. Selective and iron-independent ferroptosis in cancer cells induced by manipulation of mitochondrial fatty acid oxidation. *Biomaterials*, 2025, 320:123259.	12.8
Mitochondria	ATP/ADP	Li S, Shan Z, Zhao G, et al. Impaired mitochondria-initiated crosstalk with lysosomes reciprocally aggravates mitochondrial defect through LManVI. *Nature Communications*, 2025, 16(1):7304.	15.7
	ECAR	Wang X, Qin G, Yang J, Zhao C, Ren J, Qu X. A subcellular selective APEX2-based proximity labeling used for identifying mitochondrial G-quadruplex DNA binding proteins. *Nucleic Acids Research*, 2025, Jan 7;53(1):gkae1259.	16.6
	Mitochondrial Complex I	Deng Y, Hou M, Wu Y, et al. SIRT3-PINK1-PKM2 axis prevents osteoarthritis via mitochondrial renewal and metabolic switch. *Bone Research*, 2025, 13(1):36.	14.3
	OCR	Shi Z, Zhao J, Lv Q, et al. Chemotherapeutic drug-triggered AEP-cleaved G3BP1 orchestrates stress granules/nucleoli/mitochondria in osteosarcoma. *Bone Research*, 2025, 13(1):74.	15.0
Tumor metabolic reprogramming	Glutamine	Wang K, Li J, Zhang H, et al. Tobacco Smoking Rewires Cell Metabolism by Inducing GAPDH Succinylation to Promote Lung Cancer Progression. *Cancer Research*, 2025, 85(15):2838-2857.	12.5
	NAD+/NADH	Mao L, Xarpidin B, Shi R, et al. Natural Enzyme-Loaded Polymeric Stealth Coating-Armed Engineered Probiotics by Disrupting Tumor Lactate Homeostasis to Synergistic Metabolism-Immuno-Enzyme Dynamic Therapy. *Advanced Science (Weinh)*, 2025, Apr;12(16):e2417172.	14.3

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	E-BC-F171	Glycogen Fluorometric Assay Kit (Glycosidase Method)
	E-BC-K011-M	Glucose-6-phosphate (G6P) Colorimetric Assay Kit
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	E-BC-K130-M	Pyruvic Acid Colorimetric Assay Kit
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	E-BC-K770-M	Phosphoglucose Isomerase (PGI) Activity Colorimetric Assay Kit
	E-BC-K790-M	Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity Assay Kit
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	E-BC-F067	Fatty Acid Uptake Fluorometric Assay Kit
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	E-BC-K835-M	Cell Mitochondrial Complex II Activity Assay Kit
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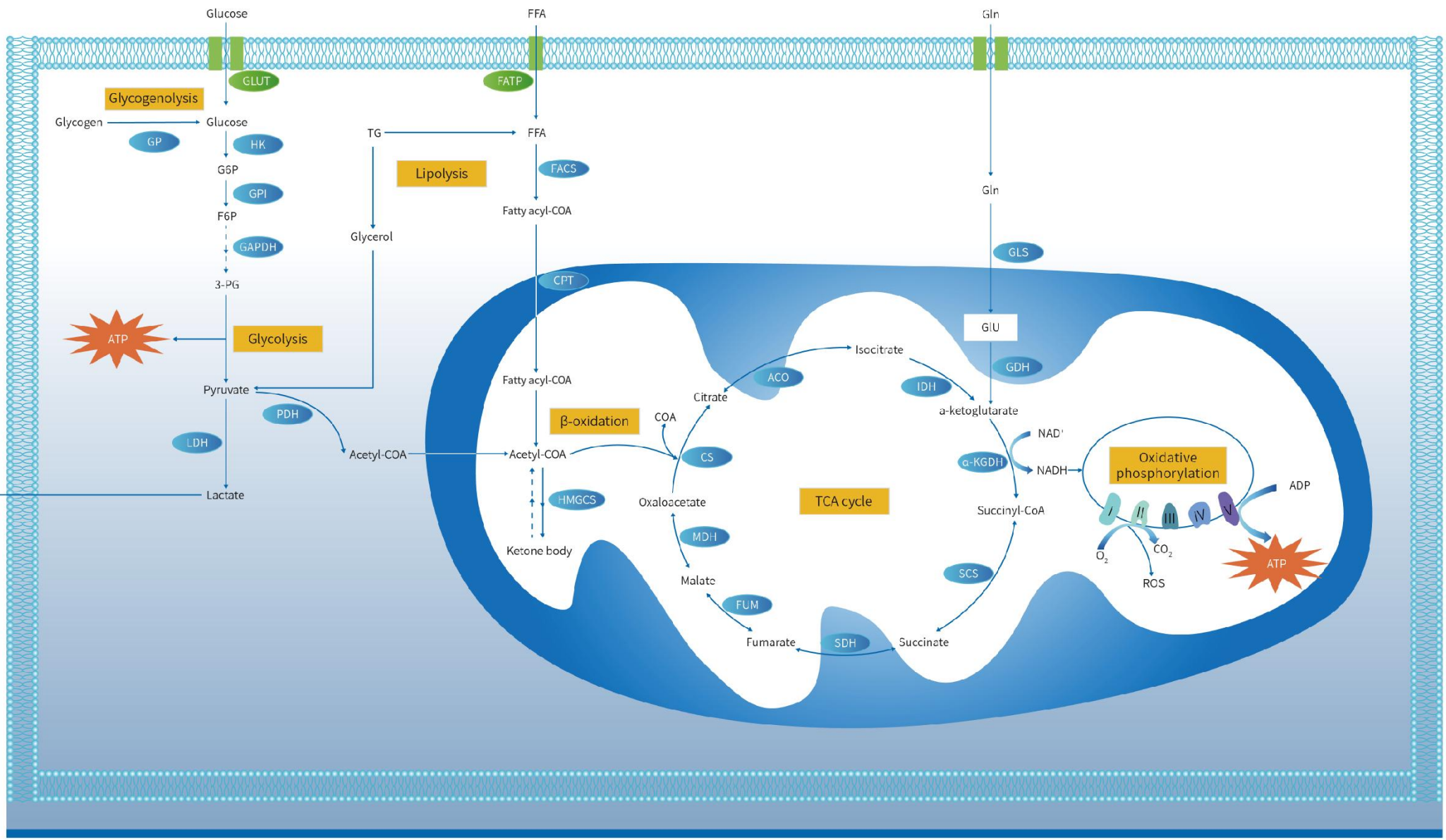

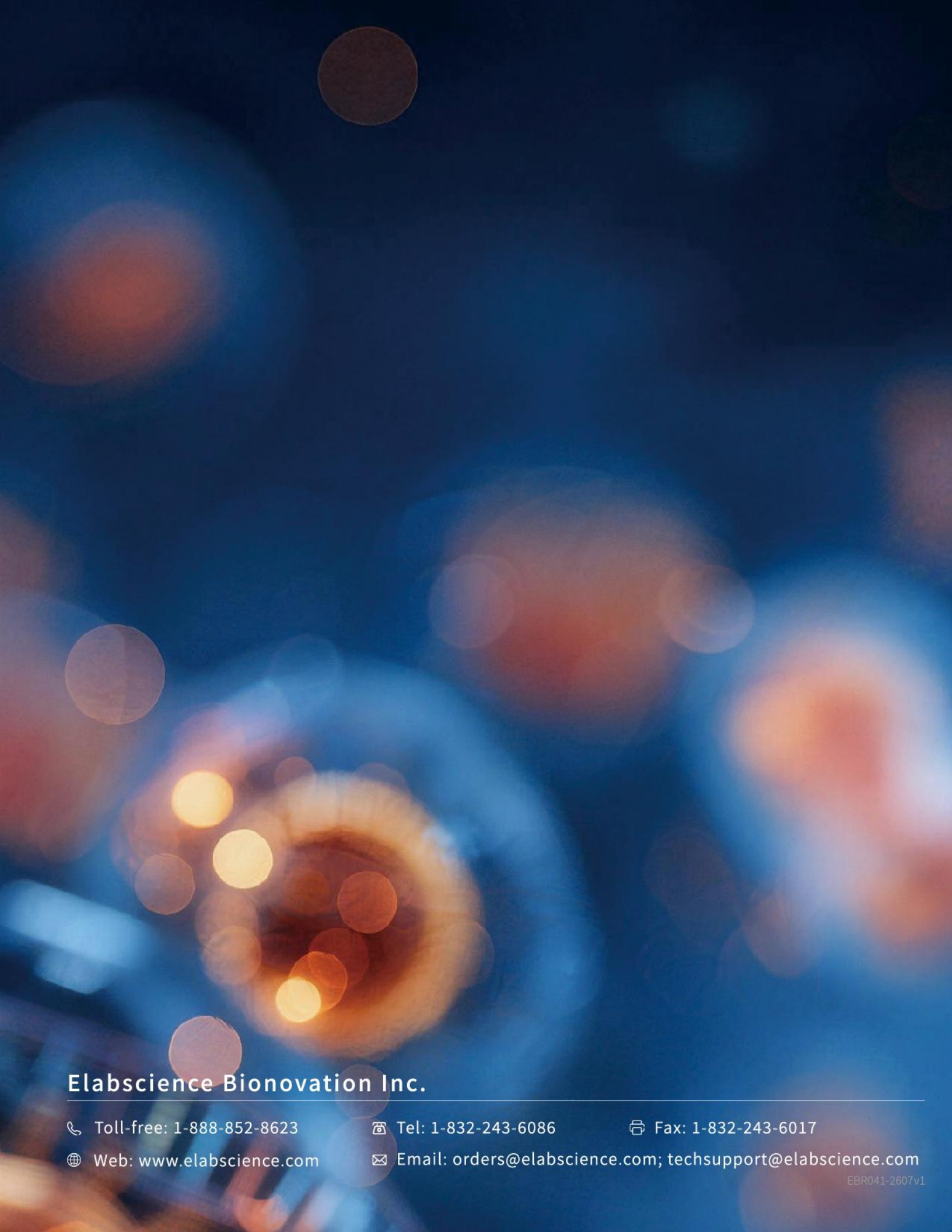

Elabscience Bionovation Inc.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

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