Metabolism & Cell Assay Guide

ASSAY GUIDE

ASPIRE TO INNOVATE

AssayGenie

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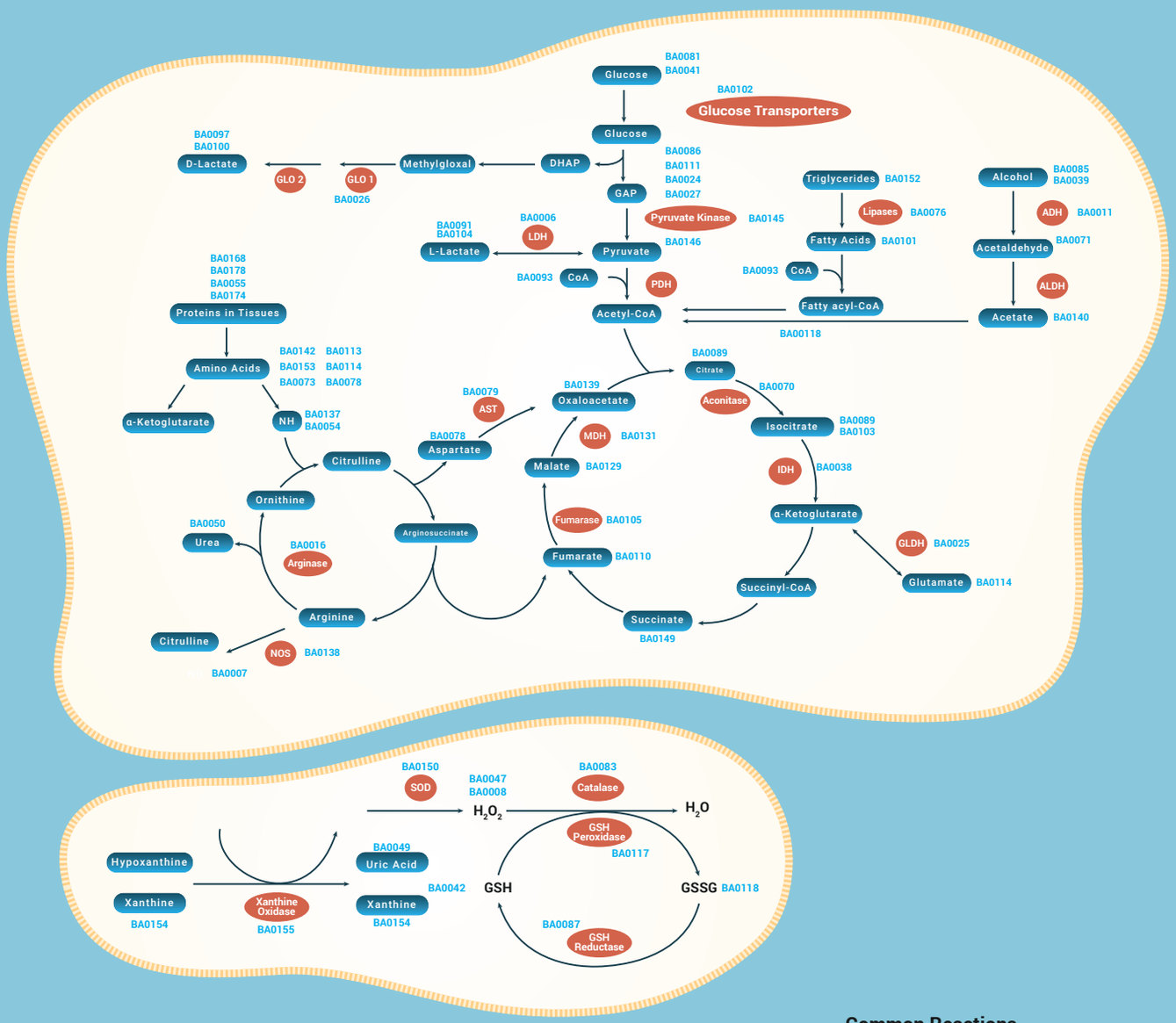


Common Reactions

BA0067 BA0106 **NAD+** ⟷ **NADH** BA0067 BA0106

BA0092 BA0107 **NADP+** ⟷ **NADPH** BA0092 BA0107

BA0063 BA0072 **ADP** ⟷ **ATP** BA0080 BA0127

BA0017 ATPase

GDP ⟷ **GTP**

BA0017 GTPase

CORE **TECHNOLOGIES**

GENIE RED

Genie Red is structurally related to resazurin. It reacts with H_2O_2 in a 1:1 stoichiometry to produce the same by-product, resorufin.

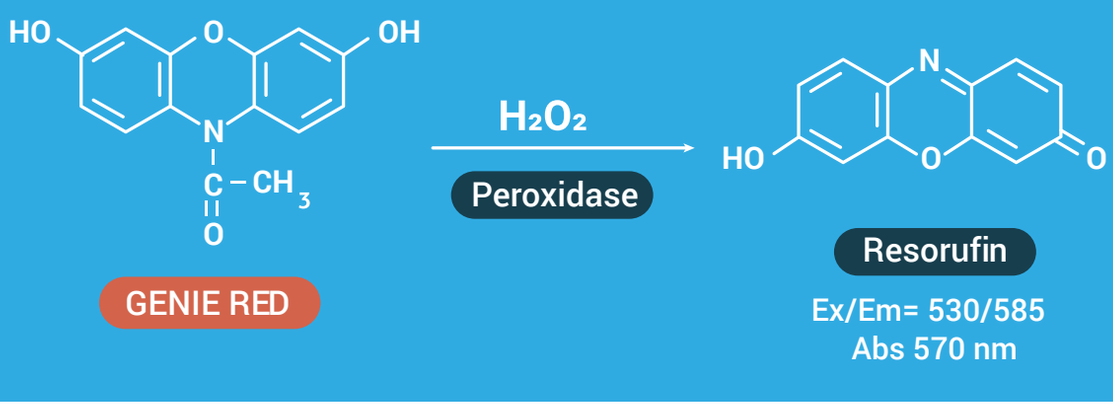
H_2O_2 is produced by a wide number of cellular and metabolic processes especially via oxidase enzymes. By coupling the production of H_2O_2 to a molecular probe, one can determine the activity of the enzyme via fluorescence or absorbance.

GENIE RED CAN BE USED TO:

- Directly assay for oxidases
- Assay for pathway products that include oxidase reactions

Genie Red is an excellent reagent for HTS applications. Not only is it robust, but it is also highly sensitive. It is flexible and can be read via absorbance or fluorescence. It is simple to use, often in 1-2 step formats and easily automatable on robotic platforms. It offers low background and is up to X10 more sensitive than other technologies.

GENIE **RED**



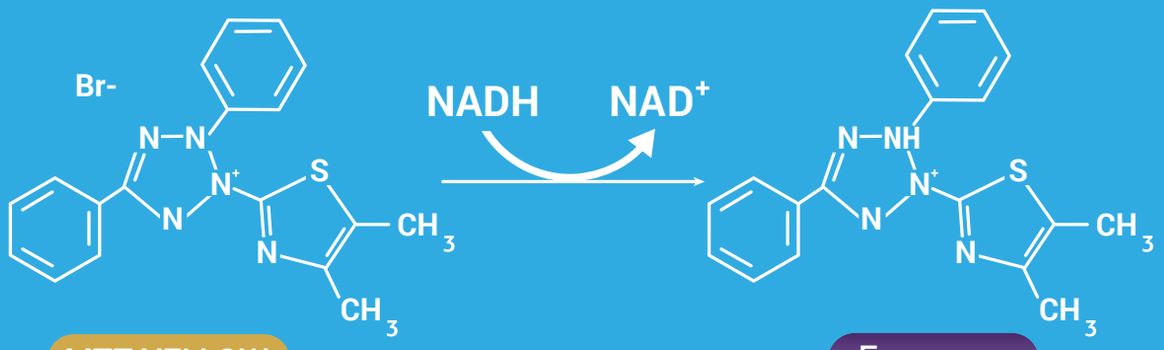
TETRAZOLIUM DYES (MTT/WST)

NADH is produced in the cell as a by-product of various enzymatic reactions. NADH is oxidised to NAD+ and reduces MTT to Formazan (by a mitochondrial reductase) which is proportional to the enzymatic activity. MTT and water soluble tetrazolium (WST) dyes such as MTS and WST-1 are excellent for HTS applications. They are simple to use, often in 1-2 step formats and easily automatable on robotic platforms.

TETRAZOLIUM DYES CAN BE USED TO:

- Measure metabolic processes involving redox reactions
- Assay for pathway products that include dehydrogenases

TETRAZOLIUM **DYES**



NADH Direct Assays

The reduction of NAD+ to NADH following the addition of a substrate is used in many assays as a direct measurement for an analyte in a sample. The amount of NADH produced is directly proportional to the concentration of analyte in the sample.

Cell Signalling, Viability, Cytotoxicity **and Apoptosis Assays**

CELL SIGNALLING

Cell signalling generally refers to the processes that govern and co-ordinate the activities of the cell. Cell signalling pathways enable the cell to respond and adapt to changes in its microenvironment and provides a basis for initiating cellular responses such as tissue repair, homeostasis and immunity. Alterations to cellular signalling pathways are often responsible for the development of wide variety of diseases such as cancer, diabetes and auto-immunity.

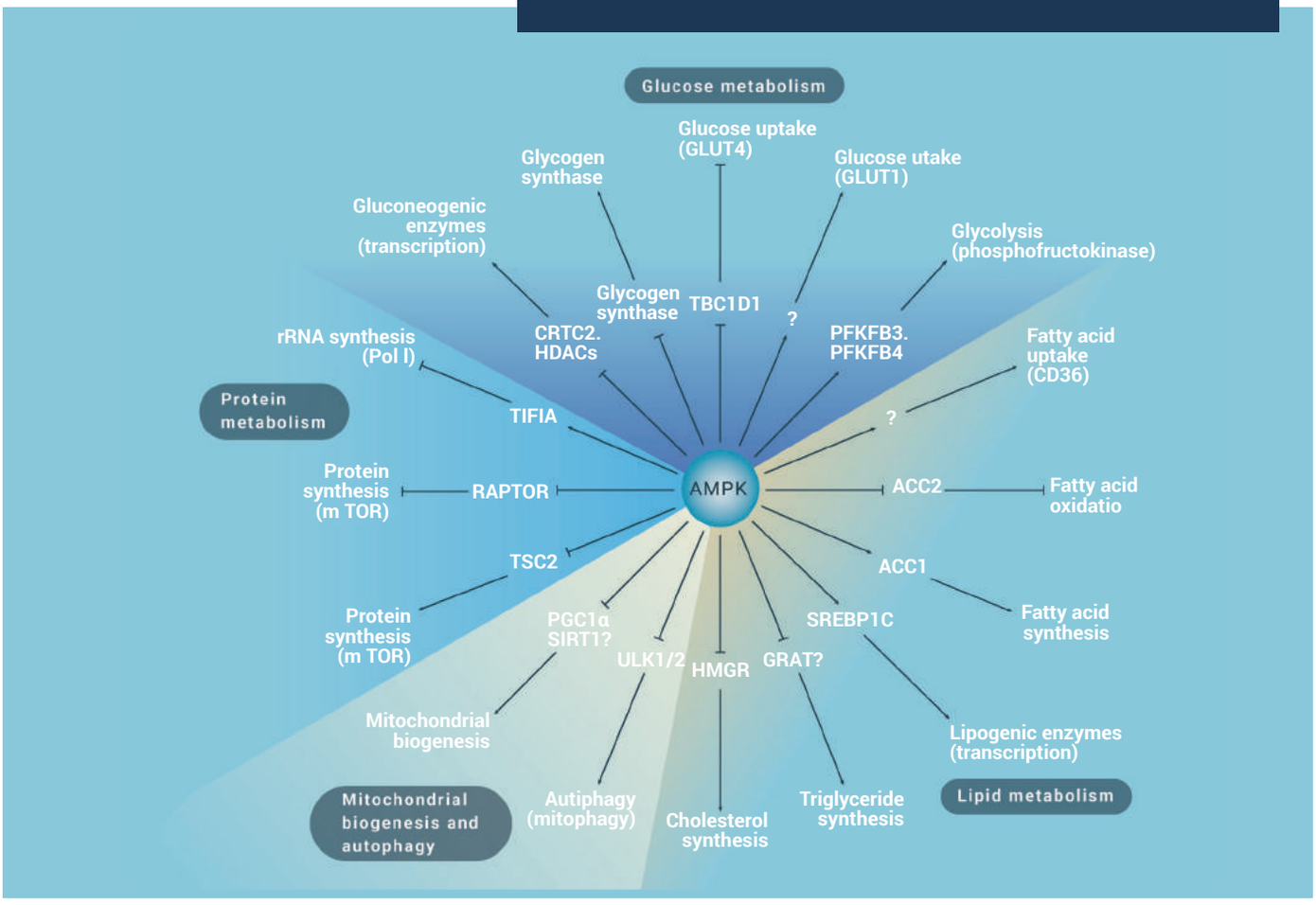
In order to understand cellular processes, it is necessary to understand the signalling pathways which initiate and drive such processes. Signalling pathways consist of a number of proteins and secondary messengers which translate extracellular information into a cellular response. Cellular process such as proliferation, differentiation and apoptosis are controlled in this way.

Signalling pathways can be activated in a number of ways the most common of which is through the binding of extracellular ligands such as growth factors and cytokines to a receptor. Kinases and phosphatases play key roles in many signalling cascades.

AMP-activated protein kinase (AMPK) is an essential cellular energy sensor, activated in response to decreasing energy levels. Upon activation AMPK promotes ATP production through the expression of proteins involved catabolism and switching off biosynthetic pathways in order to conserve ATP.

Additionally the ERK phosphorylation cascade has been deemed important for intracellular signalling such as the Krebs cycle. The ERK signalling cascade functions in a wide variety of cellular functions including proliferation and differentiation. The ERK phosphorylation cascade is linked to many upstream signalling proteins with oncogenic potential.

AMPK SIGNALLING PATHWAYS



CELL SIGNALLING ASSAYS

PRODUCT	READOUT	SAMPLES	ABSORBANCE	MECHANISM
AMPK Phosphorylation Assay Kit (BA0075)	Fluorometric	Cell tissue etc	FL360/450nm, 530/585nm	In-cell ELISA
ERK Phosphorylation Assay Kit (BA0098)	Fluorometric	Cell tissue etc	FL360/450nm, 530/585nm	In-cell ELISA
Kinase Assay Kit (BA0125)	Fluorometric	All kinases	FL530/585nm	Genie red
NFkB Phosphorylation Assay Kit (BA0136)	Fluorometric	Cell tissue etc	FL360/450nm, 530/585nm	In-cell ELISA.

CELL SIGNALLING | ATP ASSAYS

PRODUCT	READOUT	SAMPLES	DETECTION	MECHANISM
ATPase/GTPase Assay Kit (BA0017)	Colorimetric	ATPase, GTPase	OD620nm	Improved malachite green reagent
ATP Assay Kit (BA0080)	Luminescent	Cells etc	N/A	Luminescence
ADP/ATP Ratio Assay Kit (BA0127)	Luminescent	Cells etc	N/A	Luminescence

CELL VIABILITY AND CYTOTOXICITY

Different assays are required for the identification of necrosis and apoptosis. Necrosis is measured through changes in cell membrane integrity, which normally occur rapidly. During apoptosis however, cell membrane integrity is maintained for a longer period. Cellular cytotoxicity can be detected through the detection of cytosolic enzymes such as LDH which have been released into the surrounding cell culture as a result of necrosis and loss of membrane integrity.

Cell viability assays can determine the effect a drug or potential therapeutic compound may have on cell division and survival. Cell cytotoxicity leads to a reduction in cell viability and initiates cell death through necrosis and/or apoptosis.

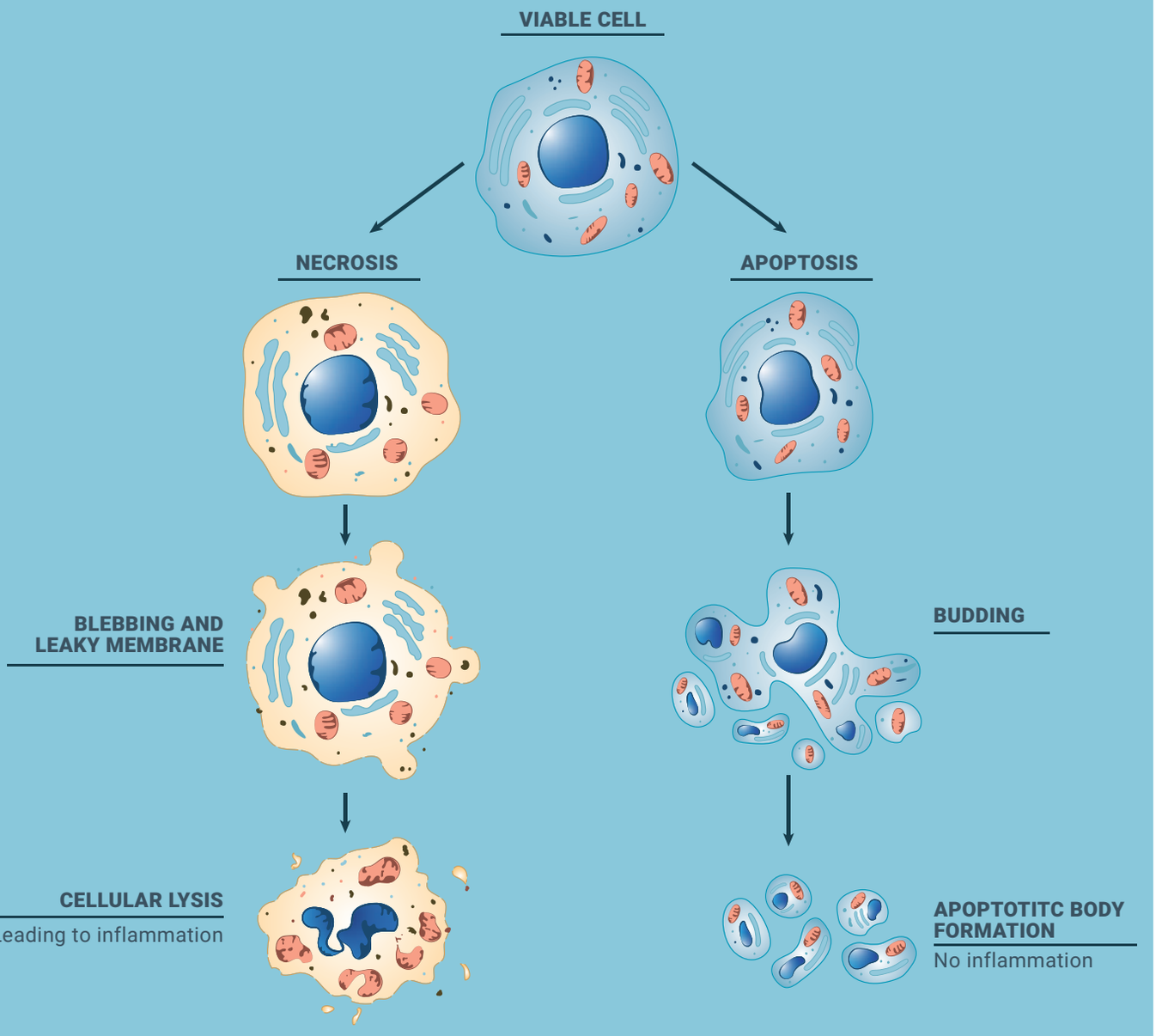
Unlike necrosis, apoptosis is set in motion by extrinsic and intrinsic factors. A unique feature of apoptotic cells is caspase activation and DNA fragmentation, during this process the cell membrane remains intact. Apoptosis doesn't trigger an immune response, unlike necrosis which activates danger associated molecular patterns (DAMPs). Caspases play a key role in initiating and driving apoptosis. Caspase-3/7 is a primary effector caspase activated by caspase 8 and 9 which trigger apoptosis through proteolysis of antiapoptotic proteins (ICAD, BCL-1, PARP etc). Detection of caspases make it possible for apoptosis to be analysed in detail.

Investigation and determination of cell cytotoxicity, viability, apoptosis and inflamation are among the most important methods in biological research. Such assays aid in development of new therapeutic agents and the identification of potential drug targets.

CELL VIABILITY AND CYTOTOXICITY ASSAYS

PRODUCT	READOUT	SAMPLES	DETECTION	MECHANISM
Caspase-3/7 Activity Assay Kit (BA0020)	Fluorometric	Cell and tissue lysate	FL400/490nm	Specific substrate (N-Ac-DEVD-AFC) is cleaved
Cell Viability Assay (BA0002, BA0003)	Colorimetric/ Fluorometric	Cell culture	OD570nm, FL530/590nm	Genie Blue
Cytotoxicity Control Reagent (Saponin) (BA0005)	N/A	Reagent	N/A	N/A
LDH Cytotoxicity Assay Kit (BA0001)	Colorimetric	Cell culture	OD500nm	MTT
MTT Cell Viability Assay (BA0004)	Colorimetric/ Fluorometric	Cell culture	OD570nm, FL530/590	MTT

APOPTOSIS AND NEOCROSIS **PATHWAYS**



Apoptosis & Necrosis Pathway: Necrosis is uncontrolled cell death caused by external factors such as infection. During Necrosis, membrane blebbing occurs followed by leakage and lysis. Apoptosis, or programmed cell death, is a mechanism of controlled targeted cell death. During this process, small blebs form, DNA breaks apart and membrane budding gradually occurs. This leads to apoptotic body formation that contain organelles, DNA and other cellular components.

LUCIFERASE **ASSAY**

Luciferases are a class of oxidative enzymes found in a number of species which enables them to emit light or 'bioluminescence', the most well-known example being the firefly luciferase. Light is emitted via a chemical reaction in which luciferin is converted to oxyluciferin by the luciferase enzyme, the energy generated from this reaction is released in the form of light. This reaction is highly energetically efficient, as nearly all the energy put into the reaction is rapidly converted to light, making it an extremely sensitive reporter assay.

$$\text{Luciferin} + O_2 + \text{ATP} \xrightarrow{\text{Luciferase}} \text{Oxyluciferin} + CO_2 + \text{AMP} + \text{PPi} + \text{Light}$$

A luciferase reporter assay can be used to study gene expression at a transcriptional level, for measuring or tracking the expression of a cloned gene. It widely used as it is convenient, relatively inexpensive and gives quantitative measurements instantaneously.

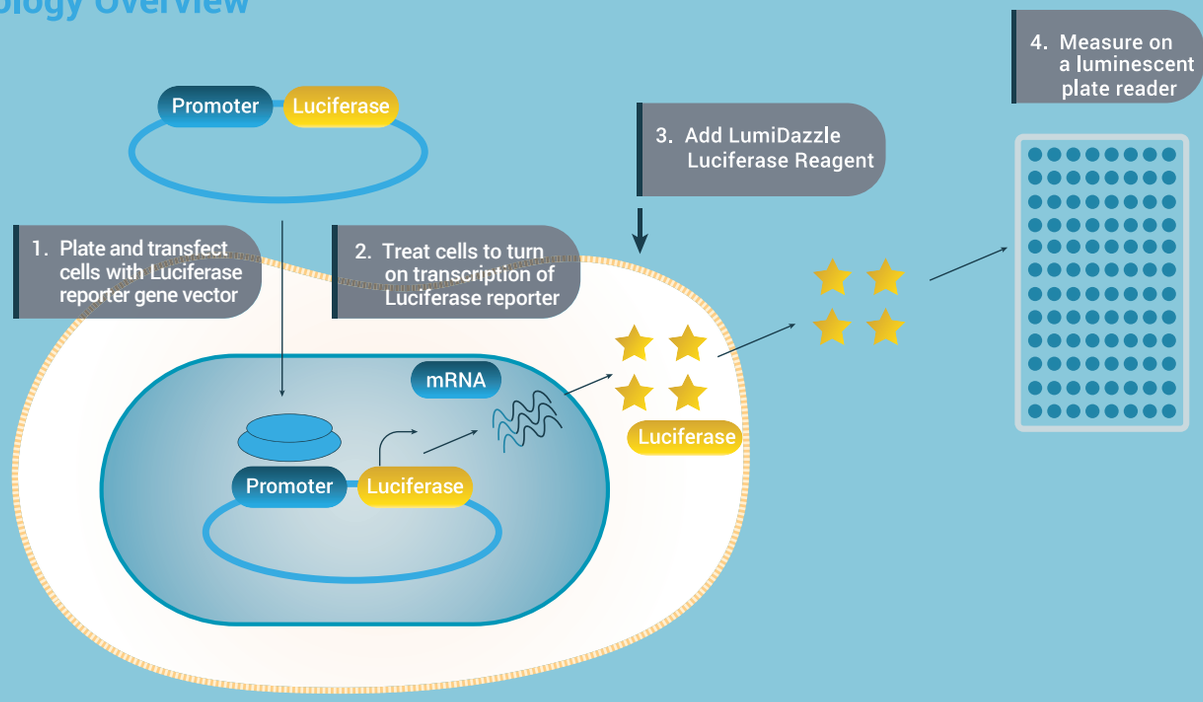
A dual luciferase assay in which secondary bioluminescence takes place is highly recommended. A dual luciferase assay enables normalisation of luciferase expression to a control and determination of relative transfection efficiency.

LUCIFERASE **ASSAYS**

PRODUCT	READOUT	SAMPLES
Dual Luciferase Reporter Gene Assay Kit (BA0180)	Lumiescent	Adherent Cells
Luciferase Reporter Gene Assay Kit (BA0181, 182,183)	Lumiescent	Adherent Cells

LUCIFERASE **REPORTER**

Technology Overview



Luciferase reporter Assay: 1) Cells are plated and transfected with either a luciferase reporter vector alone or together with a control Renilla vector. 2) Cells are treated to turn on the appropriate signalling pathway thus leading to expression of the Luciferase. 3) Luciferin containing reagents are added which fuel the luciferase and lead to the production of light that is directly proportional to the amount of luciferase expressed.

PHOSPHATE AND PYROPHOSPHATE ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Acid Phosphatase Activity Assay Kit (BA0010)	Colorimetric	Biological (e.g. serum) samples	OD405nm	p-Nitrophenol
Acid Phosphatase Activity Assay Kit (BA0156)	Fluorometric	Biological (e.g. serum) sample	FL360/450	MUP
Alkaline Phosphatase Activity Assay Kit (BA0013)	Fluorometric	Serum, plasma etc	OD405nm	p-Nitrophenyl phosphate
Alkaline Phosphatase Activity Assay Kit (BA0170)	Fluorometric	Serum, plasma etc	FL360/450nm	MUP
ALP Detection Reagent (BA0169)	Fluorometric	Biological (e.g. serum) samples	FL360/450nm	MUP
Malachite Green Assay (BA0164)	Colorimetric	Biological, environment etc	OD620nm	Malachite Green
Phosphate Assay Kit (BA0165)	Colorimetric	Enzyme reactions with free phosphate	OD620nm	Malachite Green
pNPP Phosphatase Assay Kit (500 tests) (BA0167)	Colorimetric	Protein phosphatases	OD405nm	pNPP
pNPP Phosphatase Assay Kit (1000 tests) (BA0166)	Colorimetric	Protein phosphatases	OD405nm	pNPP
Sodium Orthovanadate - Phosphatase Inhibitor (BA0163)	Colorimetric	Reagent	N/A	N/A

ENERGY **METABOLISM**

CELL ENERGY METABOLISM

The uptake and metabolism of nutrients is central to generation of energy and biomass. Energy for cellular activities is derived from organic compounds, driving the formation of ATP from ADP and inorganic phosphate.

GLYCOLYSIS

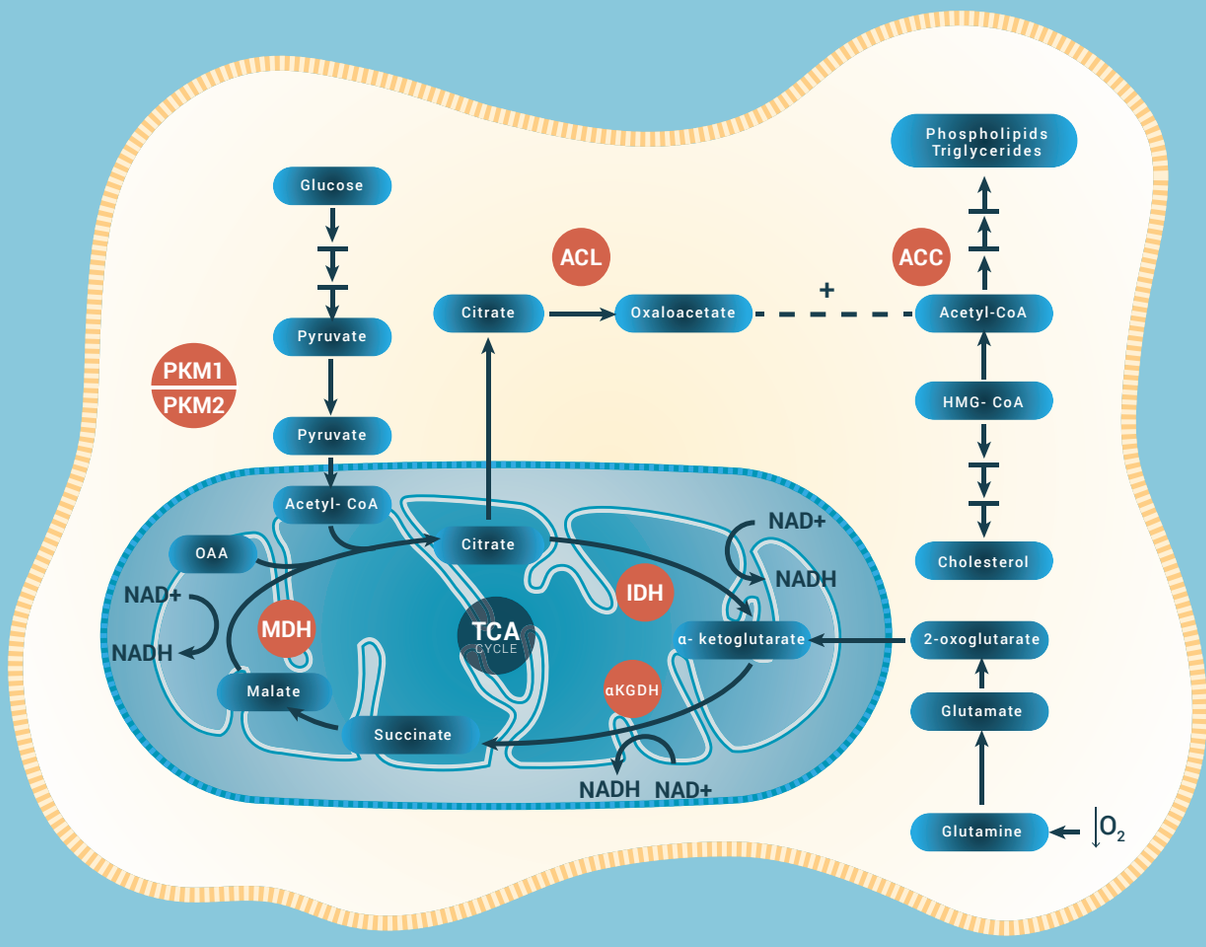
During glycolysis glucose in catabolized into pyruvate, amino acids and fatty acids which feed into the TCA cycle/Krebs cycle resulting in CO_2 oxidation and the generation of ATP through oxidative phosphorylation.

THE WARBURG EFFECT

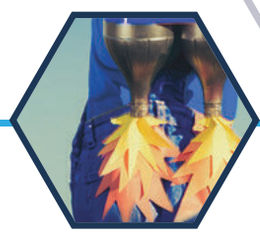
A feature of many cancerous cells and other rapidly proliferating cells is the Warburg effect or aerobic glycolysis, whereby glycolysis is uncoupled from the TCA cycle/Krebs cycle in the presence of oxygen. Some cancers can also bypass the reliance on glucose by taking up endogenous acetate to synthesize acetyl CoA. Decreased oxygen levels, commonly seen in hypoxic environments such as cancer, promote lipid uptake, glucose import, glycolysis, glutamine uptake and glutaminolysis.

The metabolic needs of a cell can vary greatly depending on cell cycle stage, with proliferating cells having greater energy demands. Transformed cancer cells also have increased energy demands for nutrients such as glucose and glutamine in order to support macromolecular synthesis.

OVERVIEW OF GLYCOLYSIS, TCA CYCLE AND LIPID SYNTHESIS PATHWAY.



Glycolysis, the tricarboxylic acid (TCA) cycle and lipid synthesis pathway overview.
ACC: acetyl-CoA carboxylase; ACL: ATP citrate lyase; FASN: fatty acid synthase; F1, 6PP, fructose 1,6 bisphosphatase; F6P, frutose-6-phosphate; G3P, glyceraldehyde 3 phosphate; G6P, glucose 6 phosphate; HMG-CoA, 3 hydroxy 3 methylglutaryl-CoA; HMGCR: HMG CoA reductase; OAA, OAA, oxaloacetate; PKM: pyruvate kinase M; ROS, reactive oxygen species; TCA, tricarboxylic acid.



NAD+, NADH, NADP & NADPH **ASSAYS**

Nicotinamide adenine dinucleotides such as (NAD+, NADH, NADP+ and NADPH) are fundamental to cellular energy metabolism. NAD+, NADH, NADP+ and NADPH are known to be essential for the maintenance of cellular redox potential and macromolecule biosynthesis.

Signalling pathways which include NAD+, NADH, NADP+ and NADPH have been shown to regulate cancer development, DNA repair and cell cycle progression. As a result of this NAD+, NADH, NADP+ and NADPH are important targets, capable of linking the metabolic state of cells with energy homeostasis and gene regulation.

NAD+, NADH, NADP+ & NADPH ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Coenzyme A (CoA) Assay Kit (BA0093)	Colorimetric/ Fluorometric	Biological	OD570nm, FL530/585nm	Genie Red
NAD/NADH Assay Kit (BA0067)	Colorimetric	Cell or tissue extracts	OD565nm	MTT
NAD/NADH Assay Kit (BA0106)	Fluorometric	Cell or tissue extracts etc	FL530/585nm	Genie Red
NADP/NADPH Assay Kit (BA0092)	Colorimetric	Cell or tissue extracts etc	OD565nm	MTT
NADP/NADPH Assay Kit (BA0107)	Fluorometric	Cell or tissue extracts etc	FL530/585nm	Genie Red

ENERGY METABOLISM ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Maltose Assay Kit (BA0132)	Colorimetric/ Fluorometric	Serum, urine, food and beverages, etc	OD570nm, FL530/585nm	Genie Red
Oxalate Assay Kit (BA0141)	Colorimetric	Urine, animal and plant tissue samples	OD595nm	Genie Red
Oxaloacetate Assay Kit (BA0139)	Colorimetric/ Fluorometric	Plasma, serum, tissue and culture media	OD570nm, FL530/585nm	Genie Red
Pyruvate Assay Kit (BA0146)	Colorimetric/ Fluorometric	Biological	OD570nm, FL530/585nm	Genie Red
Pyruvate Kinase Activity Assay Kit (BA0145)	Colorimetric/ Fluorometric	Plasma, serum, cell and tissue etc	OD570nm, FL530/585nm	Genie Red
Sialic Acid 1-Step Assay Kit (BA0148)	Colorimetric/ Fluorometric	Biological	OD570nm, FL530/585nm	Genie Red
Sialic Acid Assay Kit (BA0058)	Colorimetric/ Fluorometric	Serum, plasma, saliva, milk etc	OD549nm, FL555/585nm	Warren Method
Succinate Assay Kit (BA0149)	Colorimetric/ Fluorometric	Food, beverage, agricultural products, and other biological samples	OD570nm, FL530/585nm	Genie Red
Sucrose Assay Kit (BA0151)	Colorimetric	Serum, plasma, urine,saliva, milk, culture medium, food, beverage, agriculture etc	OD565nm	MTT

ENERGY METABOLISM ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Glucose Uptake Assay Kit (BA0102)	Fluorometric	Cell culture	FL530/585nm	2-DG
Glucose-6-Phosphate (G6P) Assay Kit (BA0111)	Fluorometric	Cell culture	OD460nm	WST
Glucose-6-Phosphate Dehydrogenase Activity Assay (BA0027)	Colorimetric	Plasma, serum, tissue and culture media, etc	OD565nm	MTT
Glycerol Assay Kit (BA0115)	Colorimetric/ Fluorometric	Biological, food, beverage etc	OD570nm, FL530/585nm	Genie Red
Glycogen Assay Kit (BA0065)	Colorimetric/ Fluorometric	Serum, plasma etc	OD570nm, FL530/585nm	Genie Red
Glycolysis Assay Kit (BA0086)	Colorimetric	Cell culture	OD565nm	MTT
Invertase Activity Kit (BA0123)	Colorimetric/ Fluorometric	Biological, environment (soil) etc	OD570nm, FL530/585nm	Genie Red
Malate Assay Kit (BA0129)	Colorimetric	Malate in food, juice, beverage and other agricultural products	OD565nm	MTT
Malate Dehydrogenase Assay (BA0131)	Colorimetric	Biological samples (e.g.plasma, serum, erythrocytes, tissue and culture media)	OD565nm	MTT

ENERGY METABOLISM ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
D-Mannitol Assay Kit (BA0133)	Colorimetric	Biological, food, beverage and agricultural products	OD565nm	MTT
Choline Assay Kit (BA0088)	Colorimetric/ Fluorometric	**Serum, plasma, urine, saliva, milk, tissue, cells etc**	OD570nm, FL530/585nm	Genie Red
Fructose Assay Kit (BA0109)	Colorimetric	Serum, plasma, saliva, urine, milk, culture medium, food, beverage, agriculture etc	OD565nm	MTT
Fumarase Activity Assay Kit (BA0105)	Colorimetric	Biological samples (e.g. plasma, serum, erythrocytes,tissue and culture media)	OD565nm	MTT
Fumarate Assay Kit (BA0110)	Colorimetric	Food, beverage and other biological samples (e.g. cell lysate, tissue homogenate, serum)	OD565nm	MTT
Galactose Assay Kit (BA0112)	Colorimetric/ Fluorometric	Serum, plasma, urine, saliva, milk, culture medium, food, beverage, agriculture etc	OD570nm, FL530/585nm	Genie Red
Glucose Assay Kit (BA0041)	Colorimetric	Serum, plasma, urine,saliva, milk, culture medium,food, agriculture etc	OD630nm (Chemical)	Improved o-Toluidine method
Glucose Assay Kit (BA0081)	Colorimetric/ Fluorometric	Serum, plasma, urine, saliva, milk, culture medium, food, agriculture etc	OD570nm, FL530/585nm	Genie Red
Glucose Dehydrogenase Assay Kit (BA0024)	Colorimetric	Biological samples (e.g. plasma, serum, tissue and culture media)	OD565nm	MTT
Glucose Oxidase Assay Kit (BA0116)	Colorimetric/ Fluorometric	Cell/tissue lysate, cell culture media etc	OD570nm, FL530/585nm	Genie Red

ENERGY METABOLISM ASSAYS

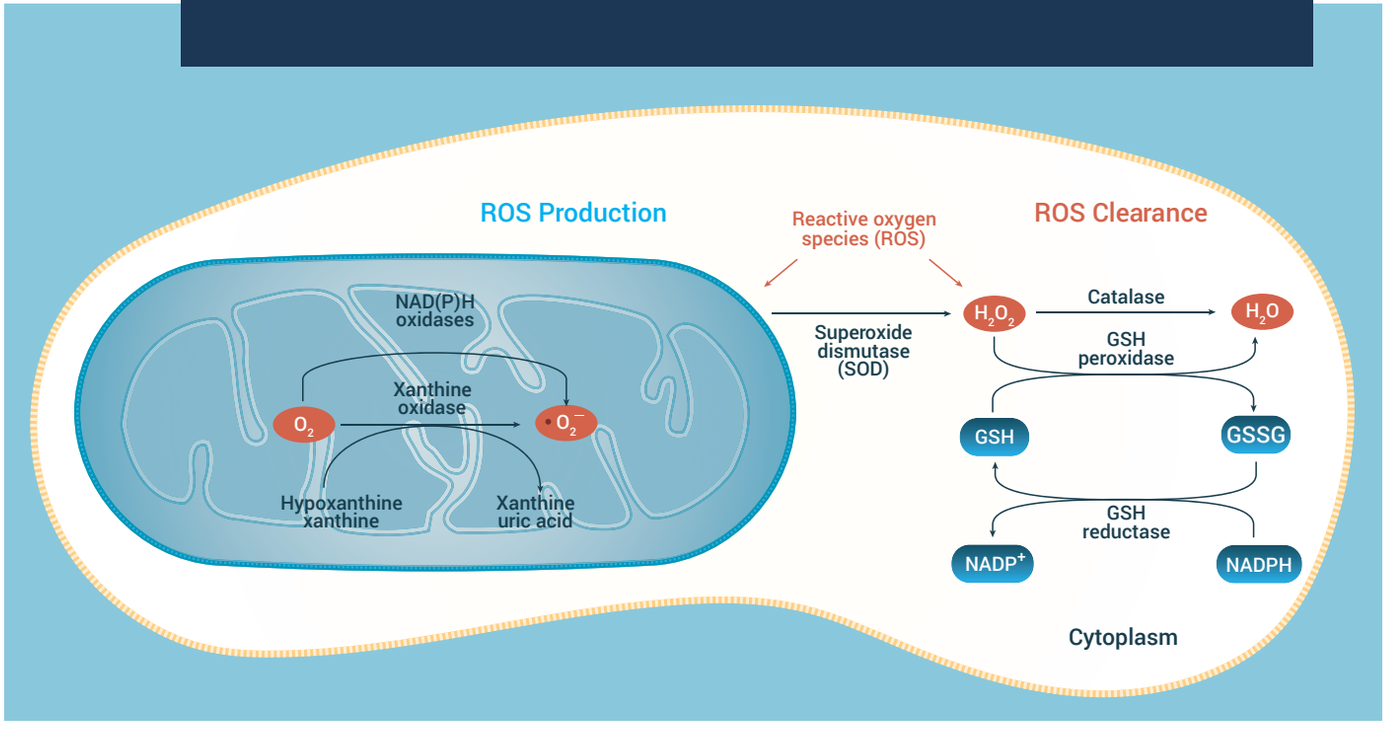
PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Acetate Assay Kit (BA0140)	Colorimetric/ Fluorometric	Serum, plasma, food, agriculture and environment	OD570nm, FL530/585nm	Genie Red
Acetylcholine Assay Kit (BA0069)	Colorimetric/ Fluorometric	Serum, plasma, urine, saliva, milk, tissue, and cell culture etc	OD570nm, FL530/585nm	Genie Red
Aconitase Activity Assay Kit (BA0070)	Colorimetric	Biological samples (e.g. cell lysate, tissue homogenate, serum etc)	OD565nm	MTT
alpha-Amylase Assay Kit (BA0082)	Colorimetric	Blood, saliva, urine, agriculture etc	OD585nm	Genie Red
alpha-Glucosidase Activity Assay Kit (BA0012)	Colorimetric	Biological	OD405nm	PNG
alpha-L-Fucosidase Assay Kit (AFU) (BA0023)	Colorimetric	Plasma, serum, tissue, celllysate etc	OD405nm	PNG
alpha-Mannosidase Activity Assay Kit (BA0014)	Colorimetric	Biological samples (e.g. plasma, serum, tissue and culture media)	OD405nm	PNG
beta-Hydroxybutyrate (Ketone Body) Assay Kit (BA0124)	Colorimetric	Serum, plasma, urine etc	OD340nm	NADH Direct

OXIDATIVE **STRESS**

Cellular oxidative stress can be identified through increased activity of reactive oxygen species (ROS) as a result of mitochondrial dysfunction and a decrease in the effectiveness of antioxidant defences such as glutathione. Increased oxidative stress can result in apoptosis and has been shown to associate with a variety of diseases such as atherosclerosis, stroke, Parkinson's and Alzheimer's dsease.

ROS although a natural by-product of normal metabolism, under oxidative stress conditions levels of ROS can increase dramatically. ROS are generated following the reduction of molecular oxygen to superoxide (O2-). Dismutation (simultaneous oxidation and reduction) of superoxide (O2-) produces H_2O_2, which can be partially reduced to hydroxyl radical (OH) or fully reduced to water. Exogenous sources of ROS production include ionizing radiation, smoke and certain drugs.

MITOCHONDRIAL DYSFUNCTION - **ROS AND ANTIOXIDANTS**



Assay Genie offers a number of assays for determining mitochondrial dysfunction via the production of ROS and Antioxidants.

Oxidative Stress | ROS AND Oxidative stress assays

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
MPO Activity Assay Kit (BA0134)	Fluorometric	Cell lysates, tissues, etc	FL530/585nm	Genie Red
Nitric Oxide Assay Kit (BA0007)	Colorimetric	Plasma, serum, urine tissue/cells and foods	OD540nm	Improved Greiss Method
Nitric Oxide Synthase (NOS) Activity Assay Kit (BA0138)	Colorimetric	Biological	OD540nm	Improved Greiss Method
Nitric Oxide Synthase (NOS) Inhibitor Screening Assay Kit (BA0121)	Colorimetric	Nitric Oxide Synthase	OD540nm	Improved Greiss Method
Peroxidase Activity Assay Kit (BA0008)	Colorimetric/ Fluorometric	Biological	OD570nm, FL530/585nm	Genie Red
Peroxide Activity Assay Kit (BA0047)	Colorimetric	Serum, citrate-plasma, urine, cell lysate, culture media etc	OD585nm	Xylenol Orange
Superoxide Dismutase Activity Assay Kit (BA0150)	Colorimetric	Blood, cell, tissue etc	OD440nm	WST
Xanthine Assay Kit (BA0154)	Colorimetric/ Fluorometric	Cell lysate, serum, and other biological samples	OD570nm, FL530/585nm	Genie Red
Xanthine Oxidase Assay Kit (BA0155)	Colorimetric/ Fluorometric	Whole blood, plasma serum, urine, tissue and cell extracts	OD570nm, FL530/585nm	Genie Red

OXIDATIVE SRESS | GLUTATHIONE AND ANTIOXIDANTS

Glutathione is an important cellular antioxidant. It is involved in phase II biotransformation, the breakdown and processing of harmful agents to the cell. Glutathione can occur as a reduced monomer (GSH) or an oxidised dimer (GSSG) acting to protect against oxidative stress. 98% of glutathione in the body is found in its reduced form (GSH).

GLUTATHIONE AND ANTIOXIDANT ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Antioxidant Assay Kit (BA0059)	Colorimetric	Serum, plasma, urine, saliva, food and beverage etc	OD570nm	Cu^{2+} reduced to CU
Catalase Activity Assay Kit (BA0083)	Colorimetric/ Fluorometric	Serum, plasma, urine, saliva, cell culture etc	OD570nm, FL530/585nm	Genie Red
Glutahione Peroxidase (GPX) Activity Assay Kit (BA0117)	Colorimetric	Biological	OD340nm	NADH Direct
Glutathione Assay Kit (BA0042)	Colorimetric	Whole blood, plasma, serum, urine, tissue and cell extracts	OD412nm	DTNB (Ellmans Reagent)
Glutathione S-transferase (GST) Activity Assay Kit (BA0028)	Colorimetric	Biological samples (e.g. cell lysates, tissues, etc)	OD340nm	CDNB
GSH/GSSG Assay Kit (BA0118)	Colorimetric	Whole blood, plasma, serum, urine, tissue and cell extracts	OD412nm	DTNB (Ellmans reagent)

ANCILLARY **ASSAYS**

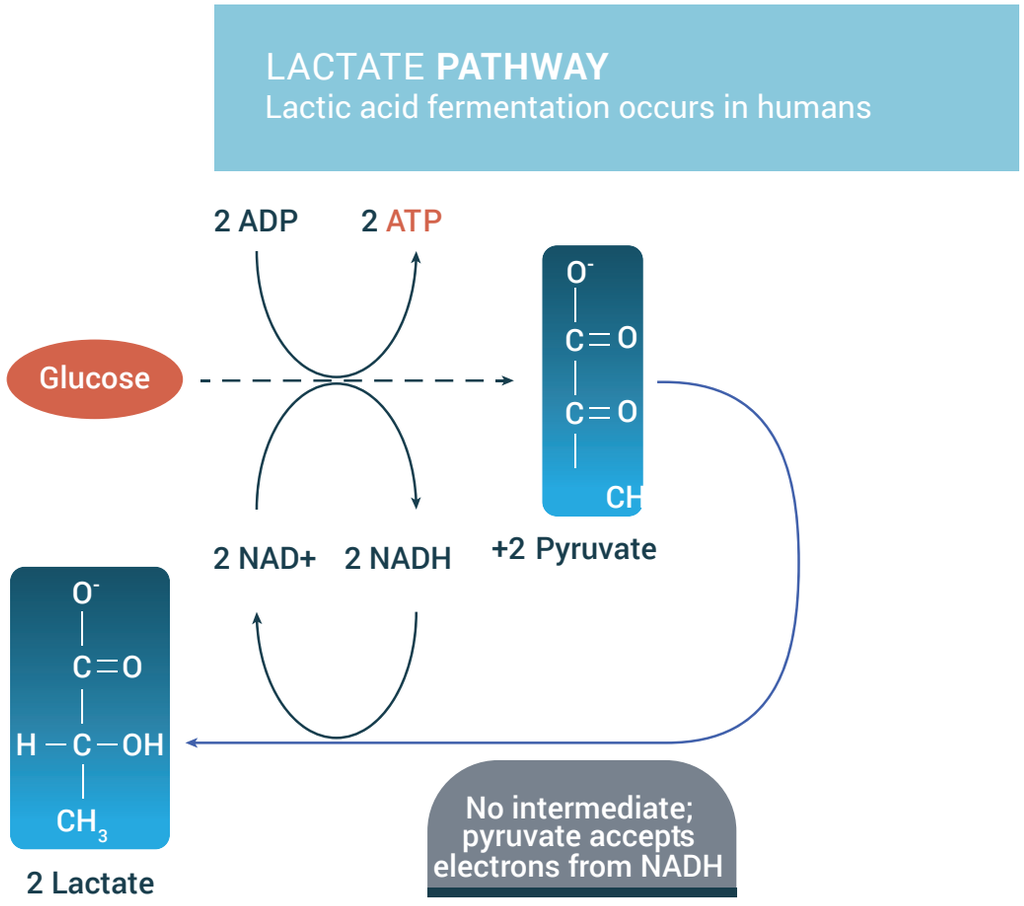
PRODUCT	READOUT	SAMPLES	DETECTION	MECHANISM
Acetylcholinesterase Inhibitor Assay Kit (BA0160)	Colorimetric	Acetylcholinesterase	OD412nm	Improved DTNB, Ellmans Method
Bradford Protein Assay (BA0168)	Colorimetric	Biological, food, agriculture etc	OD595nm	Improved Coomassie Blue G Method
DNA Assay kit (BA0171)	Fluorometric	Plasmid DNA, genomic DNA, cDNA, DNA following polymerase chain reaction, and DNA extracted from gel and other matrices	FL340/450nm	Hoechst dye
Metaphosphoric Acid (BA0162)	Chemical Reagent	For sample deproteination	N/A	N/A
Total Protein (Urine/CSF) Assay Kit (BA0178,179)	Colorimetric	Urine, cerebrospinal fluid etc	OD600nm	Improved pyrogallol red-molybdate protei dye-binding assay.
Total Protein Assay Kit (BA0174)	Fluorometric	Biological	FL360/450nm	Improved o-phthalaldehyde Method

ANAEROBIC RESPIRATION & LACTATE PRODUCTION

Anaerobic respiration is cellular respiration which occurs in the cytoplasm in the absence of oxygen. It is less efficient and produces less ATP in comparison to aerobic respiration. The final electron acceptor in aerobic respiration is oxygen. During anaerobic respiration less oxidising terminal electron acceptors are favoured such as sulphate, nitrate, sulphur and fumarate. These have a smaller reduction potential when compared to oxygen and therefore result in less energy being produced per oxidised molecule.

During anaerobic respiration, particularity during exercise, L-Lactate is produced from pyruvate via the activity of the enzyme lactate dehydrogenase (LDH). LDH does not increase in concentration until the rate of production exceeds the rate of lactate removal. This is governed by a variety of factors including the oxidative capacity of the tissue, concentration and isoform of LDH and monocarboxylate transporters.

In yeast, anaerobic respiration occurs when acetaldehyde is reduced to ethanol generating NAD+.

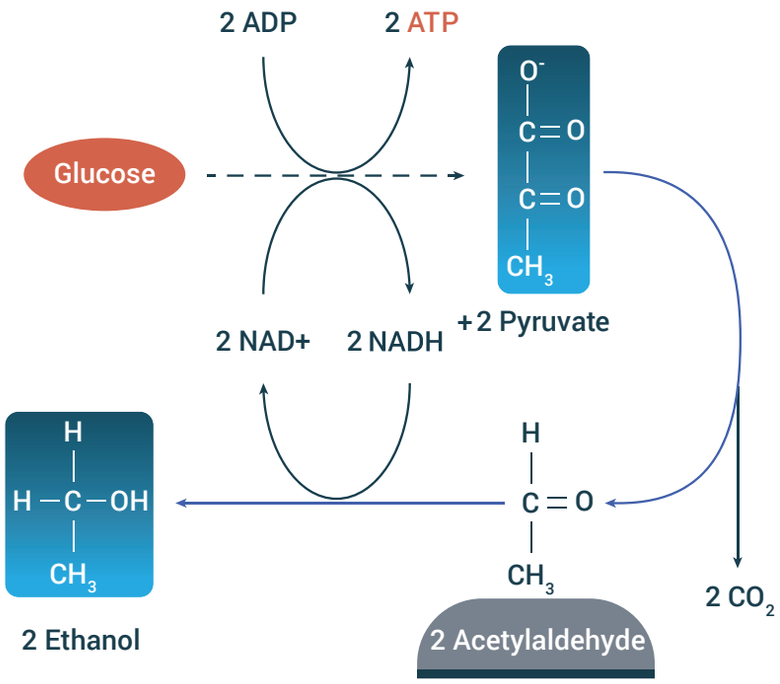


Anaerobic Metabolism Pathway: When oxygen supply is insufficient, Pyruvate is converted to Lactate by Lactate Dehydrogenase. During this process NAD+ is regenerated to power the production of ATP.

ENERGY METABOLISM | LACTATE PRODUCTION ASSAYS

PRODUCT	READOUT	SAMPLES	DETECTION	MECHANISM
D-Lactate Assay Kit (BA0097)	Colorimetric	Serum, plasma, cell culture media etc	OD565nm	MTT
D-Lactate Assay Kit (BA0100)	Fluorometric	Serum, plasma, cell culture media etc	FL530/585nm	Genie Red
Lactate Dehydrogenase Assay (BA0006)	Colorimetric	Serum, plasma etc	OD565nm	MTT
Lactose Assay Kit (BA0126)	Colorimetric/ Fluorometric	Biological (milk), food and agriculture etc	OD570nm, FL530/585nm	Genie Red
Lactulose Assay Kit (BA0128)	Colorimetric	Food, beverage, agricultural products and biological samples	OD565nm	MTT
L-Lactate Assay Kit (BA0091)	Colorimetric	Serum, plasma, cell culture media etc	OD565nm	MTT
L-Lactate Assay Kit (BA0104)	Fluorometric	Serum, plasma, cell culture media etc	FL530/585 nm	Genie Red

ETHANOL **PATHWAY**



Alcohol Fermentation Pathway: During Glycolysis, Glucose is broken down into 2 Pyruvate molecules producing ATP. The Pyruvate molecules are converted into CO2 and 2 Acetylaldehydes respectively. The latter is converted into Ethanol with concomitant NAD+ regeneration.

ENERGY METABOLISM | ALCOHOL METABOLISM ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Acetaldehyde Assay Kit (BA0071)	Colorimetric	Biological samples (e.g. plasma, serrum, urine, tissue and culture media) and food, beverage samples (e.g. wine, coffee, and juice)	OD565nm	MTT
Alcohol Dehydrogenase Activity Assay (BA0011)	Colorimetric	Biological (e.g. plasma, serum, urine, tissue and culture media)	OD565nm	MTT
D-Sorbitol Assay Kit (BA00147)	Colorimetric	Biological (e.g. blood), food, beverage and agriculture samples	OD565nm	MTT

ENERGY METABOLISM | ALCOHOL METABOLISM ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Ethanol Test Kit (BA0039)	Colorimetric	Alcoholic beverages etc	OD580nm (Chemical)	Dichromate Method
Ethanol Test kit (BA0085)	Colorimetric	Serum, plasma, urine, saliva samples etc	OD565nm (Enzymatic)	MTT
Sorbitol Dehydrogenase Activity Assay Kit (BA0056)	Colorimetric	Biological samples e.g. plasma, serum, urine, tissue and culture media	OD565nm	MTT

ENERGY METABOLISM | OTHER METABOLISM ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
B-N- Acetylglucosaminidase Activity Assay Kit (BA0053)	Colorimetric	Urine, serum, plasma, cell lysate, etc	OD405nm	p-Nitrophenol
Diamine Oxidase Activity Assay Kit (BA0172)	Fluorometric	Serum, plasma	FL530/585nm	Genie red
Formaldehyde Assay Kit (BA0022)	Fluorometric	Biological, food, beverage, environment	FL370/470nm	Formaldehyde is derivatized with acetoacetanilide in the presence of ammonia
Histamine Assay Kit (BA0120)	Colorimetric	Food, beverage and agricultural products	OD565nm	MTT
Hydrogen Peroxide Assay Kit (BA0047)	Colorimetric	Serum, citrate-plasma, urine, cell lysate, culture media etc	OD585nm	Xylenol Orange
Intestinal Permeability Assay Kit (BA0122)	Colorimetric	Urine	OD565nm	Combination of Lactulose Assay Kit and Mannitol Assay Kit
Monoamine Oxidase (MAO) Assay Kit (BA0130)	Fluorometric	Enzyme preparations	FL530/585nm	Genie red
Neuraminidase Activity Assay Kit (BA0135)	Colorimetric/ Fluorometric	Biological	OD570nm, FL530/585nm	Genie red
HRP Detection Reagent (BA0173)	Fluorometric	Biological	OD570nm, FL530/585nm	Genie Red

LIPID **METABOLISM**

LIPID METABOLISM COMPRISES OF 4 KEY STAGES:

1. Lipid synthesis

2. Lipid Uptake

3. Storage

4. Utilization

The above stages are tightly regulated especially during fasting and periods of prolonged exercise.

Fatty acid synthesis occurs in the cytoplasm as a result of the hydrophobic nature of lipids. Acetyl CoA and malonyl CoA act as substrates for fatty acid production, a reaction which is catalysed by fatty acid synthase.

When whole-body energy stores are maximal, excess glucose, fatty acids and amino acids are used in the liver to synthesize fatty acids, which are exported to White Adipose Tissue (WAT), where they are stored as triacylglycerols.

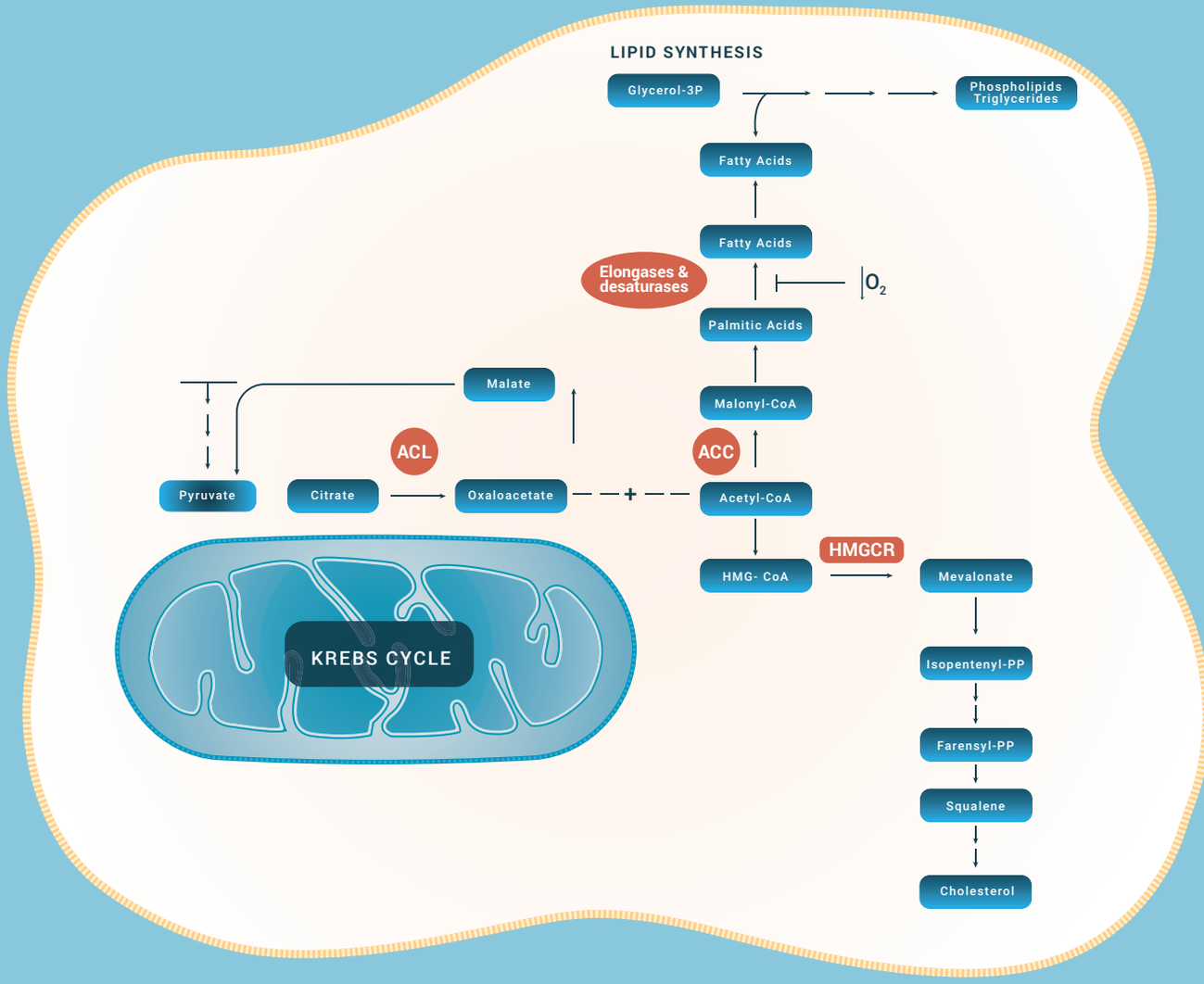
LIPID METABOLISM ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Adipolysis Assay Kit (BA0076)	Colorimetric	Cell culture media	OD570nm,	Genie Red
Cholesterol Assay Kit (BA0021)	Fluorometric	Adherent cells	FL485/535nm	Binding probe
Cholesterol Assay Kit (BA0064)	Colorimetric/ Fluorometric	Serum, plasma etc	OD570nm, FL530/585nm	Genie Red

LIPID METABOLISM ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Cholesterol Assay Kit (BA0084)	Colorimetric	Serum, plasma etc	OD340nm	NADH Direct
Free Fatty Acid Measurement Kit (BA0101)	Colorimetric/ Fluorometric	Serum, plasma, urine, saliva, milk, cell cultures, food, agriculture etc	OD570nm, FL530/585nm	Genie Red
HDL and LDL/VLDL Assay Kit (BA0066)	Colorimetric/ Fluorometric	Serum	OD570nm, FL530/585nm	Imporved PEG precipitation
HDL and LDL/VLDL Assay Kit (BA0119)	Colorimetric	Serum	OD340nm	NADH Direct
Isocitrate Assay Kit (BA0089)	Colorimetric	Food, beverage, biological samples (e.g. cell lysate, tissue homogenate, serum, etc)	OD565nm	MTT
Isocitrate Dehydrogenase Assay kit (BA0038)	Colorimetric	Plasma, serum, tissue and culture media etc	OD565nm	MTT
Lipase Assay Kit (BA0052)	Colorimetric	Serum, plasma, saliva, urine etc	OD412nm	Improved BALB Method
Phospholipid Assay Kit (BA0143)	Colorimetric/ Fluorometric	Biological	OD570nm, FL530/585nm	Genie Red
Phospholipase D Assay Kit (BA0144)	Colorimetric/ Fluorometric	Biological	OD570nm, FL530/585nm	Genie Red
TBARS Assay (BA0060)	Colorimetric/ Fluorometric	Serum, plasma, urine, saliva etc	OD535nm, FL560/585nm	TBA
Triglyceride Assay Kit (BA0152)	Colorimetric	Serum, plasma etc	OD570nm	Genie Red

Lipid & Cholesterol SYNTHESIS



Lipid & Cholesterol Synthesis Pathway: Key metabolites and enzymes involved in the lipid and cholesterol biosynthesis pathways.

BLOOD/URINE **METABOLITES**

Metabolites are the products of enzyme-catalysed reactions which occur naturally within cells and can be described as intermediates and products of metabolism. Levels of metabolites can reflect alterations in gene regulation, post-transcriptional regulation, pathway interactions, and environmental pertubations. Identifying the direct metabolite readout of a cell is an important indicator of physiological homeostasis.

To be classified as a metabolite a variety of criteria must be met:

1. The metabolite must be found inside cells.
2. The metabolite must be acted upon by enzymes.
3. The products of metabolites must be able to enter subsequent reactions.
4. Many metabolites can control the pace of metabolism.
5. Must serve useful biological functions in cells.

BLOOD/URINE METABOLITE ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Alanine Transaminase Assay Kit (BA0074)	Colorimetric	Serum, plasma etc	OD340nm	NADH Direct
Arginase Activity Assay Kit (BA0016)	Colorimetric	Enzyme preparations, serum, plasma, tissue culture etc	OD430nm	Chromagen
Ascorbic Acid Assay Kit (BA0077)	Colorimetric/ Fluorometric	Serum, plasma, urine, saliva, milk, tissue, and cell culture	OD570nm, FL530/585nm	Genie Red

BLOOD/URINE METABOLITE ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Aspartate Assay Kit (BA0078)	Colorimetric/ Fluorometric	Plasma, serum, tissue, culture media etc	OD570nm, FL530/585nm	Genie Red
AspartateTransaminase (AST) Activity Assay Kit (BA0079)	Colorimetric	Serum, plasma etc	OD340nm	NADH Direct
BCG Albumin Assay Kit (BA0029)	Colorimetric	Serum, plasma, urine, biological preparations	OD620nm	Improved Bromcresol Green
BCP Albumin Assay Kit (BA0030)	Colorimetric	Serum, plasma, urine, biological preparations	OD610nm	Improved Bromcresol Purple
Bile Acid Assay Kit (BA0099)	Fluorometric	Serum, plasma, urine and other biological samples	FL530/585nm	Improved Genie Red
Bilirubin Assay Kit (BA0031)	Colorimetric	Serum	OD530nm	Jedrassik-Grof Method
Citrate Assay Kit (BA0090)	Colorimetric/ fluorometric	Serum, plasma, agriculture etc	OD570nm, FL530/585nm	Genie Red
Creatine Assay Kit (BA0095)	Colorimetric/ Fluorometric	Serum, plasma, urine, saliva etc	OD570nm, FL530/585nm	Genie Red
Creatine Kinase Activity Assay Kit (BA0094)	Colorimetric	Serum, plasma etc	OD340nm	Improved NADH Direct
Creatinine Assay Kit (BA0034)	Colorimetric	Urine, serum, plasma and biological preparations	OD510nm	Jaffe Method

BLOOD/URINE METABOLITE ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Glutamate Dehydrogenase (GLDH) Activity Assay Kit (BA0025)	Colorimetric	Serum, plasma, cell, tissue, agriculture etc	OD565nm	MTT
Glutamine Assay Kit (BA0113)	Colorimetric	Serum, plasma, urine, cell, tissue etc	OD565nm	MTT
Heme Assay Kit (BA0044)	Colorimetric	Blood, serum, plasma, urine etc	OD400nm	Aqueous alkaline solution method
Hemoglobin Assay Kit (BA0043)	Colorimetric	Blood, serum, plasma, urine etc	OD400nm	Triton/NaOH method
Histamine Assay Kit (BA0120)	Colorimetric	Food, beverage and agricultural products	OD565nm	MTT
Indican Assay Kit (1000 tests) (BA0036)	Colorimetric	Urine	OD480nm	Improved Curzon and Walsh Method
Indican Assay Kit (100 tests) (BA0037)	Colorimetric	Urine	OD480nm	Improved Curzon and Walsh Method
Indole Assay Kit (BA0046)	Colorimetric	Bacterial growth medium	OD565nm	Modified Ehlrich's and Kovac's reagents
Isocitrate Assay Kit (BA0103)	Fluorometric	Food, beverage, biological samples (e.g. cell lysate, tissue homogenate, serum etc)	FL530/ 585nm	Genie Red

BLOOD/URINE METABOLITE ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
L-Alanine Assay Kit (BA0073)	Colorimetric/ Fluorometric	Plasma, serum, urine, tissue and culture media	OD570nm, FL530/585nm	Genie Red
Phenylalanine Assay Kit (BA0142)	Fluorometric	Serum, urine etc	FL530/585nm	Genie Red
Protein Creatinine Ratio Assay Kit (BA0055)	Colorimetric	Urine samples	OD600, OD530nm	Jaffe Method
Succinate Assay Kit (BA0149)	Colorimetric/ Fluorometric	Food, beverage, agricultural products, and other biological samples	OD570nm, FL530/585nm	Genie Red
Sulfate Assay Kit (BA0057)	Colorimetric	Serum, urine, food and environmental	OD600nm	Barium Sulfate
Tryptophan Assay Kit (BA0153)	Fluorometric	Serum	FL530/585nm	Genie Red
Urea Assay Kit (BA0050)	Colorimetric	Serum, plasma, urine, milk, cell/ tissue culture, bronchoalveolar lavage (BAL), food, beverage and enviroment	OD520nm (Chemical)	Improved Jung Method
Urease Activity Assay Kit (BA0061)	Colorimetric	Biological, environment etc	OD670nm	Berthelot Method
Uric Acid Assay Kit (BA0049)	Colorimetric	Serum, plasma, urine and other biological samples	OD590nm	Improved 2,4,6-tripyridyl-s-triazine
Whole Blood Hb Assay Kit (BA0062)	Colorimetric	Whole blood samples	OD570nm	Improved Triton/NaOH Method

CATIONS AND ANIONS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
Ammonia Assay Kit (BA0054)	Fluorometric	Biological (eg urine) and environmental samples	FL360/450nm	Improved o-phthalaldehyde Method
Ammonia Assay Kit (BA0137)	Colorimetric/ Fluorometric	Serum, plasma, urine, saliva, cell culture etc	OD340nm, FL360/450nm	NADH Direct
Calcium Assay Kit (BA0032)	Colorimetric	Biological, food and environment	OD612nm	Phenolsulphonephthalein Dye
Chloride Assay Kit (BA0033)	Colorimetric	Biological, food and environment	OD610nm	Improved Fried method
Chromium Release Assay (BA0019)	Colorimetric	Biological (serum, plasma etc), environmental (water, soil etc), food and beverage samples chromogenic dye	OD480nm	Chromagen
Copper Assay Kit (BA0035)	Colorimetric	Biological, environment, food and beverage	OD359nm	Chromagen
Formate Assay Kit (BA0108)	Colorimetric	Urine, serum etc	OD565nm	MTT

CATIONS AND ANIONS

PRODUCT	READOUT	SAMPLE TYPE	ABSORBANCE	MECHANISM
Iron Assay Kit (BA0040)	Colorimetric	Biological (e.g. serum) and environmental samples	OD590nm	Chromogen
Liquid Zinc Assay (BA0051)	Colorimetric	Serum, plasma, urine, saliva, food, beverage and environment	OD425nm	Chromogen
Magnesium Assay Kit (BA0045)	Colorimetric	Biological, food and beverage	OD500nm	Calmagite dye
Malachite Green Phosphate Assay Kit (BA0048)	Colorimetric	Serum, urine, saliva, sweat, tissue culture, food, environment etc	OD620nm	Improved Malachite Green

OTHER METABOLISM ASSAYS

PRODUCT	READOUT	SAMPLE TYPE	DETECTION	MECHANISM
B-N- Acetylglucosaminidase Activity Assay Kit (BA0053)	Colorimetric	Urine, serum, plasma, cell lysate, etc	OD405nm	p-Nitrophenol
Diamine Oxidase Activity Assay Kit (BA0172)	Fluorometric	Serum, plasma	FL530/585nm	Genie red
Formaldehyde Assay Kit (BA0022)	Fluorometric	Biological, food, beverage, environment	FL370/470nm	Formaldehyde is derivatized with acetoacetanilide in the presence of ammonia
Histamine Assay Kit (BA0120)	Colorimetric	Food, beverage and agricultural products	OD565nm	MTT
Hydrogen Peroxide Assay Kit (BA0047)	Colorimetric	Serum, citrate-plasma, urine, cell lysate, culture media etc	OD585nm	Xylenol Orange
Intestinal Permeability Assay Kit (BA0122)	Colorimetric	Urine	OD565nm	Combination of Lactulose Assay Kit and Mannitol Assay Kit
Monoamine Oxidase (MAO) Assay Kit (BA0130)	Fluorometric	Enzyme preparations	FL530/585nm	Genie red
Neuraminidase Activity Assay Kit (BA0135)	Colorimetric/ Fluorometric	Biological	OD570nm, FL530/585nm	Genie red
HRP Detection Reagent (BA0173)	Fluorometric	Biological	OD570nm, FL530/585nm	Genie Red