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POSTGRADUATE DEPARTMENT OF BIOTECHNOLOGY

STANDARD OPERATING PROCEDURES FOR LABORATORY INSTRUMENTS

SOP for UV-Visible Spectrophotometer

Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but does not cover time-resolved spectroscopic techniques. Spectrophotometry uses photometers that can measure a light beam's intensity as a function of its color (wavelength) known as spectrophotometers. Important features of spectrophotometers are spectral bandwidth, (the range of colors it can transmit through the test sample), and the percentage of sample-transmission, and the logarithmic range of sample-absorption and sometimes a percentage of reflectance measurement.

A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases. However they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200 nm - 2500 nm using different controls and calibrations. Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the photometric determination.

The larger the amount of transmitted light, the lower the noise of the obtained absorption spectrum so that a wider absorbance range can be measured. The light source uses a WI lamp (visible range) and a D2 lamp (ultraviolet range) that switches wavelength depending on the measurement. The double beam system divides the monochromatic light so that the diffraction grates into the control and the sample with a rotational mirror, guiding the light to the sample chamber. Instrument can measure wavelength ranging from 190 to 900nm with an accuracy of ± 0.1nm.

Quantitative analysis like - photometry, wavelength scan, time scan, multi-wavelength measurement can be performed in the instrument.

Operating Protocol

1. Switch on the spectrophotometer at least 20 mins before starting experiment to stabilize the instrument (light source) and base line correction is required.
2. Set the wave length range to be used for particular experiment (UV or Visible range).
3. Set the instrument to auto zero using blank solution in both the sample holders.
4. Then remove a blank from one sample holder, which is replaced by sample to be read and absorbance is measured at particular OD.

SOP for Ultracentrifuge

The ultracentrifuge is a centrifuge optimized for spinning a rotor at very high speeds, capable of generating acceleration as high as 2,000,000 (approx 19,600 km/s²). There are two kinds of ultracentrifuges, the preparative and the analytical ultracentrifuge. Both classes of instruments find important uses in molecular biology, biochemistry, and polymer science.

In an analytical ultracentrifuge, a sample being spun can be monitored in real time through an optical detection system, using ultraviolet light absorption and/or interference optical refractive index sensitive system. This allows the operator to observe the evolution of the sample concentration versus the axis of rotation profile as a result of the applied centrifugal field. With modern instrumentation, these observations are electronically digitized and stored for further mathematical analysis. Two kinds of experiments are commonly performed on these instruments: sedimentation velocity experiments and sedimentation equilibrium experiments. The kinds of information that can be obtained from an analytical ultracentrifuge include the gross shape of macromolecules, the conformational changes in macromolecules, and size distributions of macromolecular samples. For macromolecules, such as proteins, which exist in chemical equilibrium with different non-covalent complexes, the number and subunit stoichiometry of the complexes and equilibrium constant can be studied. Analytical ultracentrifugation has recently seen a rise in use because of increased ease of analysis with modern computers and the development of software, including an NIH supported software package, SedFit.

Preparative ultracentrifuges are available with a wide variety of rotors suitable for a great range of experiments. Most rotors are designed to hold tubes that contain the samples. Swinging bucket rotors allow the tubes to hang on hinges so the tubes reorient to the horizontal as the rotor initially accelerates. Fixed angle rotors are made of a single block of metal and hold the tubes in cavities bored at a predetermined angle. Zonal rotors are designed to contain a large volume of sample in a single central cavity rather than in tubes. Some zonal rotors are capable of dynamic loading and unloading of samples while the rotor is spinning at high speed. Preparative rotors

are used in biology for pelleting of fine particulate fractions, such as cellular organelles (mitochondria, microsomes, and ribosomes) and viruses. They can also be used for gradient separations, in which the tubes are filled from top to bottom with an increasing concentration of a dense substance in solution. Sucrose gradients are typically used for separation of cellular organelles. Gradients of caesium salts are used for separation of nucleic acids. After the sample has spun at high speed for sufficient time to produce the separation, the rotor is allowed to come to a smooth stop and the gradient is gently pumped out of each tube to isolate the separated components.

The maximum speed of the ultracentrifuge is 120,000 rpm.

SOP for Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. PCR is a common and often indispensable technique used in medical and biological research labs for a variety of applications. These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in forensic sciences and paternity testing); and the detection and diagnosis of infectious diseases. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase, after which the method is named, are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations.

Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase (an enzyme originally isolated from the bacterium Thermus aquaticus). This DNA polymerase enzymatically assembles a new DNA strand from DNA building-blocks, the nucleotides, by using single-stranded DNA as a template and DNA oligonucleotides (also called DNA primers), which are required for initiation of DNA synthesis. The vast majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample through a defined series of temperature steps.

In the first step, the two strands of the DNA double helix are physically separated at a high temperature in a process called DNA melting. In the second step, the temperature is lowered and the two DNA strands become templates for DNA polymerase to

selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions. Most PCR methods typically amplify DNA fragments of between 0.1 and 10 kilo base pairs (kbp), although some techniques allow for amplification of fragments up to 40 kbp in size. The amount of amplified product is determined by the available substrates in the reaction, which become limiting as the reaction progresses.

A basic PCR set up requires several components and reagents. These components include:

- DNA template that contains the DNA region (target) to be amplified.
- Two primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target.
- Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C.
- Deoxynucleoside triphosphates (dNTPs, sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building-blocks from which the DNA polymerase synthesizes a new DNA strand.
- Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- Bivalent cations, magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be utilized for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis.
- Monovalent cation potassium ions.

The PCR is commonly carried out in a reaction volume of 10–200µl in small reaction tubes (0.2–0.5 ml volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction. Many modern thermal cyclers make use of the Peltier effect, which permits both heating and cooling of the block holding the PCR tubes simply by reversing the electric current. Thin-walled reaction tubes permit favorable thermal conductivity to allow for rapid thermal equilibration. Most thermal cyclers have heated lids to prevent condensation

at the top of the reaction tube. Older thermo cyclers lacking a heated lid require a layer of oil on top of the reaction mixture or a ball of wax inside the tube.

Operating Protocol

PCR consists of a series of 20-40 repeated temperature changes, called cycles, with each cycle commonly consisting of 2-3 discrete temperature steps, usually three. The cycling is often preceded by a single temperature step at a high temperature (>90 °C), and followed by one hold at the end for final product extension or brief storage. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters. These include the enzyme used for DNA synthesis, the concentration of divalent ions and dNTPs in the reaction, and the melting temperature (Tm) of the primers.

1. Initial Denaturation: This step consists of heating the reaction to a temperature of 94–96 °C (or 98 °C if extremely thermostable polymerases are used), which is held for 1–10 minutes.
2. Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 94–98 °C for 20–90 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.
3. Annealing step: The reaction temperature is lowered to 50–65 °C for 20–90 seconds allowing annealing of the primers to the single-stranded DNA template. This temperature needs to be low enough to allow for hybridization of the primer to the strand, but high enough in order for the hybridization to be specific, i.e. the primer should only bind to a perfectly complementary part of the template. If the temperature is too low, the primer could bind imperfectly. If it is too high, the primer might not bind. Typically the annealing temperature is about ±5 °C below the Tm of the primers used. Stable DNA–DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The polymerase binds to the primer-template hybrid and begins DNA formation.

4. Elongation step: The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75–80 °C and commonly a temperature of 72 °C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand.
5. Final Extension: This single step is occasionally performed at a temperature of 70–74 °C (this is the temperature needed for optimal activity for most polymerases used in PCR) for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.
6. Hold Step: This step at 4–15 °C for an indefinite time may be employed for short-term storage of the reaction.

SOP for Shaker Refrigerated Incubator

In biology, an incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO_2) and oxygen content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology and molecular biology and are used to culture both bacterial as well as eukaryotic cells.

The simplest incubators are insulated boxes with an adjustable heater, typically temperature going up to 75 to 80 °C, though some can go slightly higher (generally to no more than 100 °C). The most commonly used temperature both for bacteria such as the frequently used *E. coli* as well as for mammalian cells is approximately 37 °C, as these organisms grow well under such conditions. For other organisms used in biological experiments, such as the budding yeast *Saccharomyces cerevisiae*, a growth temperature of 30 °C is optimal. More elaborate incubators can also include the ability to lower the temperature (via refrigeration), or the ability to control humidity or CO_2 levels. This is important in the cultivation of mammalian cells, where the relative humidity is typically >80% to prevent evaporation and a slightly acidic pH is achieved by maintaining a CO_2 level of 5%. A typical shaker incubator has a platform board that oscillates horizontally/circularly, powered by an electric motor. The liquid media to be stirred are held in beakers, jars, or Erlenmeyer flasks that are placed over the platform; or, sometimes, in test tubes or vials that are nested into holes in the plate.

Operating Protocol

1. To initially start the shaker, close the lid and turn the *ON/OFF SWITCH* on the right side panel of shaker to the ON position. During start-up, the LED DISPLAY will indicate the model of your shaker. When the shaker begins to operate, the LED DISPLAY will track the speed as it accelerates to the last entered set point. The shaking action may be started or stopped by pressing the *START/STOP* KEY on the KEYPAD.

2. Press *SELECT KEY* until the RPM INDICATOR (180rpm) is illuminated. If the display indicates that the shaker is OFF, press the *START/STOP* KEY. Press either the *UP* or *DOWN* KEY to enter SET MODE (the SET INDICATOR will illuminate).
3. Set the speed by using the *UP* or *DOWN* KEY until the desired set point is displayed. Continued pressure the *UP* or *DOWN* KEY will cause the setting to change more rapidly.
4. Press the *SELECT KEY* until the function °C INDICATOR illuminates. The temperature can be set from 5°C above ambient temperature to 60°C (non-refrigerated units) or from 4°C to 60°C (refrigerated units). Increasing or decreasing the set point is accomplished with the *UP* or *DOWN* KEY. During operation, if the temperature of the chamber is more than 1.0 °C higher or lower than the temperature set point, an alarm is triggered. This alarm consists of a flashing °C.
5. The shaker may be programmed to automatically stop after a preset time period of 0.1 to 99.9 hours.
6. Press the *SELECT* KEY until the HRS INDICATOR is illuminated. Press either the *UP or DOWN* KEY to enter the SET MODE and set the desired run time, between 0.1 and 99.9 hours. If the shaker is stopped, skip to Step below. If the shaker is already running: Press the *START/STOP* KEY. The shaker will stop and the display will read OFF. Press the *START/STOP* KEY again; the TIME INDICATOR will light and the shaker will start the timed run.

SOP for Microplate Spectrophotometer

The Microplate absorbance reader offers fast reading speeds, extensive onboard software capabilities, a pre-programmed validation protocol and a built-in printer in a compact, lightweight unit. The instrument can be used as a stand-alone reader or controlled by a PC or Microplate Manager™ Software. The wavelength range of the reader is 340–800 nm, permitting many applications, including enzyme-linked immunosorbent assays (ELISAs) with colorimetric substrates, protein assays (Bradford and Lowry), and DNA quantitation.

The Microplate reader instrument reads an entire 96-well plate in 6 seconds in Fast Read mode and performs dual-wavelength reads in 10 seconds. Kinetic reading can also be programmed with onboard software or controlled by Microplate Manager Software. The reader's onboard software is well suited for life science and clinical laboratories. The software includes security login features that allow locking and unlocking of key protocol settings by the administrator, generous memory capacity for protocol creation and storage, comprehensive curve-fit selections and extensive reporting functions. Onboard software includes pre-programmed protocols for Bio-Rad's Checkmark reader performance check plate to validate the installation qualification/ operational qualification/performance qualification (IQ/OQ/PQ) of the reader in compliance with GLP. Other life science and clinical protocols are also included, and a growing number of clinical testing kits. The reader comes with 4 standard absorbance filters: 405, 450, 490 and 655 nm. There are 4 additional filter options: 405, 540, 550, 570, 595, 630 and 750 nm. A wide-format internal thermal graphical printer saves counter space and simplifies stand-alone operation. The reader can also be used with an external printer with or without a data station.

Operating Protocol

1. Switch on the reader and allow the instrument for self calibration, which takes about 1-2 mins.
2. Then place the plates in the chamber and choose the respective filters for your application.
3. The instrument can read 96 wells within 30 sec.
4. And thermal printer attached to instrument will give the values read for the plates.

SOP for Microplate Reader

Plate readers also known as microplate readers are instruments which are used to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8x12 matrix) with a typical reaction volume between 100 to 200 µL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when high throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5-50 µL per well. Common detection modes for microplate assays are absorbance fluorescence intensity, luminescence, time-resolved fluorescence and fluorescence polarization.

Operating Protocol

Before switching the instrument on, check that all the cables are properly fitted according to the installation instructions.

1. Switch the instrument on by pressing the *START/ON* key. The instrument proceeds to perform the self diagnostics and displays "Self diagnostics passed" after it has been passed. The instrument is now ready for use.
2. Load the microplate. If the plate carrier is in, first drive the plate carrier out by pressing the *IN/OUT* key. Always insert the microplate so that the A1 corner is positioned in the top left-hand corner of the plate carrier. Define the measurement parameters in the Plate menu.
3. Press the *START/ON* key to start the measurement. View the results of the measurement. Save, export or print the results if desired.
4. To shut down the Multiskan-GO instrument-
    - Remove any plates from the instrument. Switch off the instrument after use by pressing and holding the *STOP/OFF* key for a few seconds.

SOP for Microcentrifuge

Centrifugation is a process which involves the use of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge, used in industry and in laboratory settings. This process is used to separate two immiscible liquids. More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force on a test tube so as to more rapidly and completely cause the precipitate ("pellet") to gather on the bottom of the tube. The remaining solution is properly called the "supernate" or "supernatant liquid". The supernatant liquid is then either quickly decanted from the tube without disturbing the precipitate, or withdrawn with a Pasteur pipette. The rate of centrifugation is specified by the angular velocity measured in revolutions per minute (RPM), or acceleration expressed as **g**. The conversion factor between RPM and **g** depends on the radius of the sample in the centrifuge rotor. The particles settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity. In the chemical and food industries, special centrifuges can process a continuous stream of particle-laden liquid.

Microcentrifuges are used to process small volumes of biological molecules, cells, or nuclei. Microcentrifuge tubes generally hold 0.5 - 2.0 ml of liquid, and are spun at maximum angular speeds of 12,000–13,000 rpm. Microcentrifuges are small enough to fit on a table-top and have rotors that can quickly change speeds. And also temperature controlled during the spinning process i.e with refrigeration facility.

Operating Protocol

1. Switch on the centrifuge and allow the instrument for few seconds to internal checking. After checking the instrument will display the current value mode and temperature of the chamber. The speed and run time shown is *0*.

2. Open the centrifuge lid and make sure that the rotor chamber is clean. The thread and the O-ring on the motor shaft must be in perfect condition.

3. The sample tubes must be kept symmetrically inside the rotor. And then close the lid gently.

4. Set the run parameters by entering the required speed (RPM- *max 12,000)*, time (Min) and temperature (°C). Press the "*Start key*" and the rotating light in the speed display indicates that the centrifuge is running.

5. As soon as the speed is '0', the display reads "*End*". By pressing the "*Open Lid*" key, you can now open the lid and you can remove your centrifugation samples. Turn Off the power switch.

SOP for High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography), is a technique in analytic chemistry used to separate the components in a mixture, to identify each component, and to quantify each component. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. HPLC has been used for medical (e.g. detecting vitamin D levels in blood serum), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and manufacturing (e.g. during the production process of pharmaceutical and biological products) purposes.

Chromatography can be described as a mass transfer process involving adsorption. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components. The active component of the column, the sorbent, is typically a granular material made of solid particles (e.g. silica, polymers, etc.), 2–50 micrometers in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the sorbent particles. The pressurized liquid is typically a mixture of solvents (e.g. water, acetonitrile and/or methanol) and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and sorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination thereof.

HPLC is distinguished from traditional ("low pressure") liquid chromatography because operational pressures are significantly higher (50–350 bar), while ordinary

liquid chromatography typically relies on the force of gravity to pass the mobile phase through the column. Due to the small sample amount separated in analytical HPLC, typical column dimensions are 2.1–4.6 mm diameter, and 30–250 mm length. Also HPLC columns are made with smaller sorbent particles (2–50 micrometer in average particle size). This gives HPLC superior resolving power when separating mixtures, which is why it is a popular chromatographic technique.

The schematic of an HPLC instrument typically includes a sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in a HPLC instrument can mix multiple solvents together in ratios changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/Vis, photodiode array (PDA) or based on mass spectrometry. Most HPLC instruments also have a column oven that allows for adjusting the temperature the separation is performed at.

The sample mixture to be separated and analyzed is introduced, in a discrete small volume (typically microliters), into the stream of mobile phase percolating through the column. The components of the sample move through the column at different velocities, which are function of specific physical interactions with the sorbent (also called stationary phase). The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called its retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte.

Many different types of columns are available, filled with sorbents varying in particle size, and in the nature of their surface ("surface chemistry"). The use of smaller particle size packing materials requires the use of higher operational pressure ("backpressure") and typically improves chromatographic resolution (i.e. the degree

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of separation between consecutive analytes emerging from the column). In terms of surface chemistry, sorbent particles may be hydrophobic or polar in nature. Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol). Some HPLC techniques use water-free mobile phases (see Normal-phase chromatography below). The aqueous component of the mobile phase may contain acids (such as formic, phosphoric or trifluoroacetic acid) or salts to assist in the separation of the sample components. The composition of the mobile phase may be kept constant ("isocratic elution mode") or varied ("gradient elution mode") during the chromatographic analysis. Isocratic elution is typically effective in the separation of sample components that are not very different in their affinity for the stationary phase. In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength. The eluting strength of the mobile phase is reflected by analyte retention times with high eluting strength producing fast elution (=short retention times). A typical gradient profile in reversed phase chromatography might start at 5% acetonitrile (in water or aqueous buffer) and progress linearly to 95% acetonitrile over 5–25 minutes. Periods of constant mobile phase composition may be part of any gradient profile. For example, the mobile phase composition may be kept constant at 5% acetonitrile for 1–3 min, followed by a linear change up to 95% acetonitrile.

The chosen composition of the mobile phase (also called eluent) depends on the intensity of interactions between various sample components ("analytes") and stationary phase (e.g. hydrophobic interactions in reversed-phase HPLC). Depending on their affinity for the stationary and mobile phases analytes partition between the two during the separation process taking place in the column. This partitioning process is similar to that which occurs during a liquid–liquid extraction but is continuous, not step-wise. In this example, using a water/acetonitrile gradient, more hydrophobic components will elute (come off the column) late, once the mobile phase gets more concentrated in acetonitrile (i.e. in a mobile phase of higher eluting strength). The choice of mobile phase components, additives (such as salts or acids) and gradient conditions depends on the nature of the column and sample components. Often a series of trial runs is performed with the sample in order to find the HPLC method which gives adequate separation.

SOP for Gel Doc System

A gel doc, also known as a gel documentation system, gel image system or gel imager, is equipment widely used in molecular biology laboratories for the imaging and documentation of nucleic acid and protein suspended within agarose gels or polyacrylamide. These gels are typically stained with ethidium bromide, commasive brilliant blue or other fluorophores such as SYBR Green, Sypro ruby. Generally, a gel doc includes an ultraviolet (UV) light transilluminator, a hood or a darkroom to shield external light sources and protect the user from UV exposure, and a CCD camera for image capturing. Recently produced imager models also include features to handle a variety of fluorescence and chemiluminescence with cooling cameras (-67 °C). The Universal hood II is designed to capture UV light and chemiluminescence images without using darkroom. The enclosure has built-in white light epi-illumination and UV transillumination. An optional white light conversion tray can also be used wit. A CCD camera is placed on the top of the Universal Hood II for capturing of the images. The camera comes with motorized zoom lens that allows remote adjustment of the lens control function (zoom, focus & iris). The Universal Hood II has three position filter slider that offers flexibility of using two different emission filters for fluorescent application.

Operating Protocol

1. Switch on the Gel Doc instrument.
2. Place the gel on the sample tray. Select the appropriate light source for the gels. If the gel to be visualized is agarose then it can be placed directly on the sample tray or if gel is PAGE then it has to be kept on white light conversation tray to be captured.
3. Then with help of window door the gels are adjusted and focused under camera. Using the software image is captured. The software controls the Zoom, Iris and exposure parameters.
4. The captured image is stored in PC and further image are used for determining the size and concentration of the given sample.

SOP for Calibrated Densitometer

A densitometer is a device that measures the degree of darkness (the optical density) of a photographic or semitransparent material or of a reflecting surface. The densitometer is basically a light source aimed at a photoelectric cell. It determines the density of a sample placed between the light source and the photoelectric cell from differences in the readings. Modern densitometers have the same components, but also have electronic integrated circuitry for better reading. Densitometer can scan two different types of light sources-

- Transmittance densitometers that measure transparent materials
- Reflective densitometers that measure light reflected from a surface.

Densitometer serves as one of the Molecular tools for gene study, to quantify the radioactivity of a compound such as radiolabeled DNA/RNA and also helps in densitometric analyses.

Operating Protocol

1. Switch on the densitometer by pushing the *On/Off* button. Densitometer performs the self test which last for about 90 secs during which ready indicators flashes briefly. The scanning light turns on and both indicator lights will stay on once. For optimal, performance, allow the densitometer to warm up for 5 mins after calibration.
2. Raise the Transparency module.
3. Place the sample facedown for both Transmittance/Reflection scanning on the densitometer image window.
4. Guide the top corner of the sample to the lower left (0, 0) corner of the image window.
5. Lower the transparency window.
6. Scanning and analysis of the given sample is performed using Quantity-1 software.

SOP for Fermenter or Bioreactor

Industrial fermentations are typically carried out in large tanks, called fermenters or bioreactor. Industrial fermentation is the intentional use of fermentation by microorganisms such as bacteria and fungi to make products useful to humans. Fermented products have applications as food as well as in general industry. Some commodity chemicals, such as acetic acid, citric acid, and ethanol are made by fermentation. The rate of fermentation depends on the concentration of microorganisms, cells, cellular components, and enzymes as well as temperature and pH. Product recovery always involves the concentration of the dilute solution. Nearly all commercially produced enzymes, such as lipase, invertase and rennet, are made by fermentation with genetically modified microbes. In some cases, production of biomass itself is the objective, as in the case of baker's yeast and lactic acid bacteria, starter cultures for cheese making. In general, fermentations can be divided into four types:

- Production of biomass (viable cellular material)
- Production of extracellular metabolites (chemical compounds)
- Production of intracellular components (enzymes and other proteins)
- Transformation of substrate (in which the transformed substrate is itself the product)

These types are not necessarily disjoint from each other, but provide a framework for understanding the differences in approach. The organisms used may be bacteria, yeasts, molds, animal cells, or plant cells. Special considerations are required for the specific organisms used in the fermentation, such as the dissolved oxygen level, nutrient levels, and temperature.

In most industrial fermentations, the organisms are submerged in a liquid medium; in others, such as the fermentation of cocoa beans, coffee cherries, and miso, take place on the moist surface of the medium. There are also industrial considerations related to the fermentation process. For instance, to avoid biological process contamination, the fermentation medium, air, and equipment are sterilized. Foam control can be achieved by either mechanical foam destruction or chemical anti-foaming agents. Several other

factors must be measured and controlled such as pressure, temperature, agitator shaft power, and viscosity. An important element for industrial fermentations is scale up. This is the conversion of a laboratory procedure to an industrial process. It is well established in the field of industrial microbiology that what works well at the laboratory scale may work poorly or not at all when first attempted at large scale. It is generally not possible to take fermentation conditions that have worked in the laboratory and blindly apply them to industrial-scale equipment. Although many parameters have been tested for use as scale up criteria, there is no general formula because of the variation in fermentation processes. The most important methods are the maintenance of constant power consumption per unit of broth and the maintenance of constant volumetric transfer rate. Depending on the nature of the fermentation, gas may be sparged into the fermentation medium. For aerobic fermentations, air is typically used because it is inexpensive to provide enough oxygen for cellular respiration. Anaerobic fermentations, such as the production of ethanol, typically do not require the addition of any air, and only require agitation from a mixer to keep the organisms suspended. Aerobic fermentations may be conducted in a variety of fermenters, such as a bubble column or a packed bed over which fermentation medium drips (as in the production of vinegar). Cooling is typically required, since organisms produce waste heat as part of their metabolism.

When a particular organism is introduced into a selected growth medium, the medium is inoculated with the particular organism. Growth of the inoculum does not occur immediately, but takes a little while. This is the period of adaptation, called the lag phase. Following the lag phase, the rate of growth of the organism steadily increases, for a certain period—this period is the log or exponential phase. After a certain time of exponential phase, the rate of growth slows down, due to the continuously falling concentrations of nutrients and/or a continuously increasing (accumulating) concentration of toxic substances. This phase, where the increase of the rate of growth is checked, is the deceleration phase. After the deceleration phase, growth ceases and the culture enters a stationary phase or a steady state. The biomass remains constant, except when certain accumulated chemicals in the culture lyse the cells (chemolysis). Unless other micro-organisms contaminate the culture, the chemical constitution remains unchanged. If all of the nutrients in the medium are consumed, or if the concentration of toxins is too great, the cells may become scenescent and begin to die

off. The total amount of biomass may not decrease, but the number of viable organisms will decrease. The microbes used for fermentation grow in (or on) specially designed growth medium which supplies the nutrients required by the organisms. A variety of media exists, but invariably contains a carbon source, a nitrogen source, water, salts, and micronutrients. In the production of wine, the medium is grape must. In the production of bio-ethanol, the medium may consist mostly of whatever inexpensive carbon source is available.

Carbon sources are typically sugars or other carbohydrates, although in the case of substrate transformations (such as the production of vinegar) the carbon source may be an alcohol or something else altogether. For large scale fermentations, such as those used for the production of ethanol, inexpensive sources of carbohydrates, such as molasses, corn steep liquor, sugar cane juice, or sugar beet juice are used to minimize costs. More sensitive fermentations may instead use purified glucose, sucrose, glycerol or other sugars, which reduces variation and helps ensure the purity of the final product. Organisms meant to produce enzymes such as beta galactosidase, invertase or other amylases may be fed starch to select for organisms that express the enzymes in large quantity. Fixed nitrogen sources are required for most organisms to synthesize proteins, nucleic acids and other cellular components. Depending on the enzyme capabilities of the organism, nitrogen may be provided as bulk protein, such as soy meal; as pre-digested polypeptides, such as peptone or tryptone; or as ammonia or nitrate salts. Cost is also an important factor in the choice of a nitrogen source. Phosphorus is needed for production of phospholipids in cellular membranes and for the production of nucleic acids. The amount of phosphate which must be added depends upon the composition of the broth and the needs of the organism, as well as the objective of the fermentation. For instance, some cultures will not produce secondary metabolites in the presence of phosphate.

Growth factors and trace nutrients are included in the fermentation broth for organisms incapable of producing all of the vitamins they require. Yeast extract is a common source of micronutrients and vitamins for fermentation media. Inorganic nutrients, including trace elements such as iron, zinc, copper, manganese, molybdenum and cobalt are typically present in unrefined carbon and nitrogen sources, but may have to be added when purified carbon and nitrogen sources are

used. Fermentations which produce large amounts of gas (or which require the addition of gas) will tend to form a layer of foam, since fermentation broth typically contains a variety of foam-reinforcing proteins, peptides or starches. To prevent this foam from occurring or accumulating, antifoaming agents may be added. Mineral buffering salts, such as carbonates and phosphates, may be used to stabilize pH near optimum. When metal ions are present in high concentrations, use of a chelating agent may be necessary.

SOP for Analytical Balance

An analytical balance is a class of balance designed to measure small mass in the sub-milligram range. The measuring pan of an analytical balance (0.1 mg or better) is inside a transparent enclosure with doors so that dust does not collect and so any air currents in the room do not affect the balance's operation. This enclosure is often called a draft shield. The use of a mechanically vented balance safety enclosure, which has uniquely designed acrylic airfoils, allows a smooth turbulence-free airflow that prevents balance fluctuation and the measure of mass down to 1 µg without fluctuations or loss of product. Also, the sample must be at room temperature to prevent natural convection from forming air currents inside the enclosure from causing an error in reading. Single pan mechanical substitution balance maintains consistent response throughout the useful capacity is achieved by maintaining a constant load on the balance beam, thus the fulcrum, by subtracting mass on the same side of the beam to which the sample is added.

Electronic analytical scales measure the force needed to counter the mass being measured rather than using actual masses. As such they must have calibration adjustments made to compensate for gravitational differences. They use an electromagnet to generate a force to counter the sample being measured and outputs the result by measuring the force needed to achieve balance. Such measurement device is called electromagnetic force restoration sensor.

Operating Protocol

1. Turn on the balance/scale. Allow the balance to pass self-test to be performed.
2. Place container/aluminium foil on balance/scale.
3. Press Tare to tare the balance/scale.
4. Place sample in container/aluminium foil on balance/scale and weigh (Max: 210 g).

Calibration/Adjustment

1. Tare the balance/scale (0.0 g).
2. Begin calibration. Calibration weight is displayed without weight unit (here: 1000).
3. Place the indicated calibration weight (1000.0) on the balance/scale.
4. After calibration, the calibration weight is displayed with wt. unit (= 1000.0 g).
5. Remove the calibration weight.

Care and Maintenance

Service:

Regular servicing by a Sartorius technician will extend the service life of your balance/scale and ensure its continued weighing accuracy. Sartorius can offer you service contracts, with your choice of regular maintenance intervals ranging from 1 month to 2 years. The optimum maintenance interval depends on the operating conditions at the place of installation and on the individual tolerance requirements.

Repairs:

Repair work must be performed by trained service technicians. Any attempt by untrained persons to perform repairs may lead to hazards for the user.

Cleaning:

Unplug the AC adapter from the wall outlet (mains supply). If you have an interface cable connected to the balance/scale port, unplug it from the port,

Clean the balance/scale using a piece of cloth which has been wet with a mild detergent (soap). After cleaning, wipe down the balance/scale with a soft, dry cloth.

Removing and Cleaning the Weighing Pan:

Lift up and remove the weighing pan together with the pan support by gripping them from under the shield ring. Make sure that you do not damage the weighing system in doing so.

Make sure that no liquid enters the balance/scale housing.
Do not use any aggressive cleaning agents (solvents or similar agents).

Cleaning Stainless Steel Surfaces:

Clean all stainless steel parts regularly. Remove the stainless steel weighing pan and thoroughly clean it separately. Use a damp cloth or sponge to clean any stainless steel parts on the balance/scale. Only use commercially available household cleaning agent that is suitable for use on stainless steel. Clean stainless steel surfaces by wiping them down. Then rinse thoroughly, making sure to remove all residues. Afterwards, allow the balance/scale to dry. If desired, you can apply oil to the cleaned surfaces as additional protection. Solvents are permitted for use only on stainless steel parts.