

**DEPARTMENT OF BIOCHEMISTRY**  
**FACULTY OF ARTS, SCIENCE AND HUMANITIES**  
**KARPAGAM ACADEMY OF HIGHER EDUCATION**  
*(Deemed to be University, Established Under Section 3 of UGC Act 1956)*  
Eachanari PO, Coimbatore – 641 021, India.

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**M.Phil / Ph.D– BIOCHEMISTRY**  
**(Effective from the academic year 2018 - 2019 and onwards)**

**PREAMBLE**

- The degree of Master of Philosophy [M.Phil] /Doctor of Philosophy (Ph.D) is awarded to a candidate who has submitted a thesis on the basis of original and independent research in any biochemistry field of research.
- This makes a contribution to the advancement of knowledge, which can be useful to the society.

**DEPARTMENT OF BIOCHEMISTRY**  
**FACULTY OF ARTS, SCIENCE AND HUMANITIES**  
**RESEARCH PROGRAM – M.Phil / PhD in Biochemistry**  
**(2018–2019 and onwards)**

Course code	Name of the course	Instruction hours / week	credits	Maximum Marks (100)
18RBC101	Research Methodology and Pedagogy	4	4	100
18RBC201	Advanced paper in Biochemistry	4	4	100
18RBC301A	Enzyme and Enzyme technology	4	4	100
18RBC301B	Cancer Biology and immunology			
18RBC301C	Medicinal Plants and Plant therapeutics			
18RBC301D	Clinical Biochemistry and Toxicology			
18RBC301E	Plant Molecular Biotechnology			
18RBC301F	Animal Tissue Culture			
<b>Program Total</b>		12	12	300

**Instruction hours/week: L: 4 T: 0 P: 0 Marks:100****End Semester Exam: 3 Hours****CourseObjective:****Equip the students with:**

- The availability of tools for literature search
- Usage of tools and documentation of available research
- Identification of lacunae in the literature
- Processing of data and evaluating statistical significance
- Nuances of scientific writing
- Dissemination of research data

**CourseOutcome:**

After successful completion, the students will understand

1. Scopus, Cochrane, Pubmed databases
2. Collation of available research and exportation to appropriate format
3. Methods involved in the identification of lacunae in the literature
4. How to Process data and evaluate statistical significance
5. Nuances of scientific writing
6. Dissemination of research data

**UNIT I: Research Problem**

Definition - Identification - Review of Literature – Lacunae identification-Research process - Research design –Experimental and non experimental designs- Exploratory – Diagnostic.

**UNIT II: Sampling methods**

Sampling - Population –Census - Sample – Types – Probability – Non Probability sampling – Sampling size – Sampling process – Hypothesis and its formulation. Sampling distribution – Students t test. Experimental design – CRD, RBD. Analysis of experimental results – ANOVA and its interpretation. Duncan's Multiple Range Test. Interpretation.

**UNIT III: Data Processing**

Data Collection Tools -Case studies - Interview – Questionnaire -Schedule - observation- Scaling techniques – Scale Construction – Rating scales. Hypothesis testing – Parametric and non parametric tests - Coding – Editing – Tabulation –Analysis – Correlation & regression.

**UNIT IV: Scientific writing and presentation**

Technical Writing: Scientific and technical subjects; formal and informal writings; formal writings/reports, handbooks, manuals, letters, memorandum, notices, agenda,minutes; common errors to be avoided. Microsoft Windows: Macintosh versions, Microsoft Word- Characteristics - Document statistics - Typical usage, Microsoft Excel - Basic operation – Charts - Using other

Microsoft applications - Using external data, Microsoft Power Point –power point viewer – versions – uses, Microsoft Access –Uses – Features.

### **UNIT- V: Pedagogical methods in higher education**

Objectives and role of higher education- Important characteristics of an effective lecture- Quality teaching and learning- lecture preparation- characteristics of instructional design- Methods of teaching and learning: Large group- Technique – lecture, seminar, symposium, team teaching, project, small group technique- simulation, role playing demonstration, Brain storing, Case discussion, and assignment, methods of evaluation- self evaluation, student evaluation, diagnostic testing and remedial teaching- question banking- electronic media in education:- ‘e’ learning researches- web based learning.

### **SUGGESTED READING**

1. B. Somekh & C. Lewin, (2005), Research methods in the social sciences, Vistaar Publications, New Delhi
2. Crotty, M. (1998), The Foundation of Social Research: Meaning and Perspective in the Research Process, Sage Publications, London
3. Blaikie, N. (2000), Beginning Social Research, Polity Press, Cambridge
4. V. Desai & R. B. Potter, (2006), Doing Development Research, Sage Publications, New Delhi.

**Instruction hours/week: L: 4 T: 0 P: 0 Marks:100****End Semester Exam: 3 Hours****CourseObjective:****Equip the students with:**

- Applications and limitations of antibody based assays
- Applications and limitations of fluorescence based assays
- Applications and limitations of microscopic assays
- Applications and limitations of nucleic acid analysis
- Analysis of protein using wet lab and computational methods
- Basic methodology of animal and plant tissue culture methods

**CourseOutcomes:****After successful completion, the students will understand**

1. Appropriate antibody based assays for a specific research question
2. Appropriate fluorescence based assays for a specific research question
3. Specific microscopic assays for a research question
4. Appropriate nucleic acid analysis for a specific research question
5. Usage of wet lab and computational methods for a specific research question
6. General methodology of animal and plant tissue culture methods

**UNIT I: Biochemical Techniques**

Fluorescent Antibody assay – Histochemical localization, ELISA techniques – Principles & Applications. Immuno radiometric assay – Principles & Applications  
Natural Products – Detection of bioactive molecules by gas chromatography, HPLC and HPTLC, Mass spectrometry, NMR. Emission Spectroscopy – Fluorescence, Phosphorescence and Chemiluminescence. X-Ray diffraction and Flow injection analysis.

**UNIT II: Microscopy and PCRTechniques**

Electron microscopy- transmission and scanning, Freeze fracture technique, specific staining of biological material. Flow Cytometry – Principles, Abnormal chromosome analysis, Karyotyping, Comet assay. DNA Fragmentation analysis – Microfabrication techniques and uses in biological applications. PCR methodology – Design of primers – RTPCR. PCR in genomic analysis and diagnostic applications. PFGE – Principles, techniques and applications.

**UNIT III: Bioinformatics Tools**

Biological database- DNA sequence database, protein sequence database SRS – Similarity searching, BLAST, FASTA Local and Global alignment, Multiple sequence alignment –

Phylogeny. Structure database – Secondary structure prediction. Predicting 3D folds (Threading) Visualisation tool.

#### **UNIT IV: Genomics and Proteomics Human Genome project**

History, techniques and applications: Anatomy of prokaryotic and human genome: genetic mapping and genetic markers-RFLP, Mini and micro satellite, STS and EST, SSCP, RAPD, AFLP, SNPs, Analysing gene expression- DNA micro array. Proteome analysis- 2D gel electrophoresis: protein-protein interactions- yeast two-hybrid system and protein micro arrays.

#### **UNIT V: Culture Techniques**

**Plant Tissue Culture:** Tissue culture media, composition and preparation, primary culture, callus and suspension cultures, somoclonal variation, micro propagation, organogenesis, Somatic embryogenesis, artificial seeds, Transfer and establishment of whole plants in soil, Haploidy: Protoplast fusion and somatic hybridization.

**Animal Tissue Culture:** Media - Natural media, balanced salt solution and simple media, serum and protein free chemically defined media. Primary cell culture, (chick, mouse and human biopsy) and methods of desegregations of tissues; continuous or established cell culture, tissue culture, organ culture; three dimensional culture, feeder layer, cell separation; cell synchronization; cryopreservation and revival.

#### **SUGGESTED READING**

1. Adrian Slater, Neigel Scott and Maark Fowler (2008), Plant Biotechnology – Genetic manipulation of plants, 2<sup>nd</sup> edition, Oxford University Press, New York.
2. Akay M. (2007) Genomics and Proteomics Engineering in Medicine and Biology Wiley-Interscience John Wiley & sons, Inc. Publication, USA.
3. Deepak Bhariog (2006), Fundamentals of Information Technology, 3<sup>rd</sup> Edition Excel Books India.
4. Hall, Christopher; Tews, Carey (Retrieved 7 November 2010). "Mac Office matches Windows — almost". InfoWorld. p. 117.
5. Freshney, R. I., & Freshney, M. G. (2010), Animal cell culture: a practical approach, 2nd ed. IRL Press at Oxford University Press.
6. Karp G. (2012), Cell and Molecular Biology: Concept and Experiments. John Willy, New York, USA.
7. Lodish et.al. (2013) Molecular Cell Biology, 7<sup>th</sup> edition, W.H.Freeman and Company, New York.USA.
8. Watson, J. D., Baker, T. A. & Bell, S. P. (2007). Molecular Biology of the Gene. 6th Ed. Benjamin Cummings.USA.

**Instruction hours/week: L: 4 T: 0 P: 0****Marks:100****End Semester Exam: 3 Hours****CourseObjective:****Equip the students with:**

- Techniques involved in the analysis of protein structure and function
- The applications and limitations of methods involved in isolation of enzymes
- The applications and limitations of methods involved in purification of enzymes
- Physical, Chemical and Biological methods of enzyme immobilization
- Clinical significance of enzyme analysis
- Biotechnological applications of enzymes

**CourseOutcomes:****After successful completion, the students will understand**

1. X-Ray crystallographic, Spectrophotometric analysis of enzymes
2. How to estimate the fold purity during isolation of an enzyme
3. Enzyme purification techniques
4. Physical, Chemical and Biological methods of enzyme immobilization
5. The clinical significance of enzyme analysis in analyzing the vital organ functions
6. Applications of enzymes in the area of medical and industrial biotechnology

**UNIT I: Protein and enzymes**

Protein structure, functions, compositions and conformation of proteins. Enzyme catalysis-Acid base catalysis, covalent catalysis, an example, serine proteases. Enzyme kinetics – Michaelis menton equation, Line weaver Burk plot, Hills equation, Hans plot.

**UNIT II: Isolation and purification of enzymes**

Sources of enzymes for industry, extraction of enzymes for scientific and industrial purposes. Downstream processing of enzymes, uses of soluble enzymes. Study of enzymes in aqueous biphasic systems. Factors affecting the enzyme activity -Substrate concentration, Enzyme concentration, pH, temperature etc.,

**UNIT III: Enzyme immobilization and their applications**

Techniques employed for immobilizing enzymes, kinetics of immobilized enzymes. Advantages and disadvantages in the utilization of soluble enzymes, Immobilized enzymes and immobilized cells. Different types of reactors of immobilized enzymes and their applications.

**UNITIV: Clinical analysis of enzymes**

Application of ELISA and EMIT in clinical analysis. Different types of Biosensors- potentiometric, amperometric, piezo - electric and immuno biosensors. Electro analytical applications of enzymes, Methods of coenzyme regeneration. Biochips and Biocomputers.

#### **UNIT V: Enzymes in Biotechnology**

Enzyme catalysis in organic solvents, Restriction endonucleases, DNA ligases, DNA polymerase and their uses in Biotechnology. Site directed mutagenesis, artificial enzymes, ribozymes and Abzymes and their uses.

#### **SUGGESTED READING**

1. Bommarius A.S., B.R. Riebel. 2004. Biocatalysis – Fundamentals and Applications, Wiley-VCH, Weinheim, Germany.
2. Buchholz K., V. Kasche, U.T. Bornscheuer. 2005. Biocatalysts and Enzyme Technology, Wiley-VCH, Weinheim, Germany.
3. Cook P. F., W.W. Cleland. 2007. Enzyme Kinetics and Mechanism, Garland Science Publishing, London, England and New York, USA.
4. Irwin Segel. 2004. Biochemical Calculations, John Wiley and Sons, California, USA.
5. Marangoni A.G. 2003, Enzyme Kinetics-A Modern Approach.



**Instruction hours/week: L: 4 T: 0 P: 0 Marks:100****End Semester Exam: 3 Hours****Course Objectives:****Equip the students with:**

- Etiology of cancer
- Steps involved in the progression of cancer
- Changes in the cell division during oncogenesis
- Programmed cells death
- Immune surveillance strategy
- Techniques involved in the assessment of cancer development and progression

**Course Outcomes:****After successful completion, the students will understand:**

1. The reasons for the development of cancer
2. Factors involved in the advancement of cancer
3. Targeting the cell division for the treatment of cancer
4. Targeting apoptosis for the treatment of cancer
5. Methods of enhancing immune surveillance for cancer treatment
6. Usage of appropriate techniques for assessment of cancer stages

**UNIT I: Biology of cancer**

Biology of cancer-Phenotype of a cancer cell causes of cancer-DNA tumor viruses, RNA tumor viruses, cell cycle and its control-role of protein kinases, checkpoints, kinase inhibitor and cellular response.

**UNIT II: Apoptosis**

Programmed cell death (Apoptosis)-Intracellular proteolytic cascade, cascade of caspase proteins, adapter proteins, Bcl-2, IAP family proteins, extra cellular control of cell division, tumor necrosis factor and related death signals.

**UNIT III: Genetic basis of cancer**

Genetic basis of cancer-oncogenes, tumor suppressor genes, aberrations in signaling pathways. oncogenic mutations in growth promoting proteins, Mutations causing loss of growth –inhibiting and cell cycle control, Role of carcinogens and DNA repair in cancer.

**UNIT IV: Immunology of cancer**

Immunity- Active, passive, humoral and cell mediated immunity. Therapeutic uses of cytokines and cytokine receptors. Test for lymphocyte function. B cell and T cell immuno deficiency disorder. Clinical laboratory methods for the detection of antigens and antibodies test for histocompatibility antigens, neoplasm of the immune system.

## **UNIT V: Cancer Techniques**

Techniques-FISH techniques, Real time PCR, Western blotting, ELISA assay, immunocytochemistry, immunohistochemistry, flow cytometry, fluorescent microscopy and confocal microscopy.

### **SUGGESTED READING**

1. Alberts, B. et al. (2008). Molecular Biology of the Cell. 5th Ed. Garland Publishing House. USA.
2. Benjamin Lewin (2007) Genes VIII, Prentice Hall. USA.
3. Brown T.A. (2010), Gene Cloning & DNA Analysis, 6nd Edition, Wiley-Blackwell, New York.
4. Karp G. (2012), Cell and Molecular Biology: Concept and Experiments. John Willy, New York.
5. Klug, W.S., Cummings, M.R, Spencer C.A and Palladino, M.A. (2012), Concept of Genetics, 10<sup>th</sup> Edition, Pearson Education, Singapore.
6. Lodish H., Berk A., Kaiser CA., Kriger M .,Scott M.P.,Bretscher A., Ploegh H., Matsudaira P.2008. Molecular Cell Biology, 6<sup>th</sup> edition, W.H. Freeman and Company, New York.
7. Janeway et al., 2012.Immunobiology, 8th Edition, Current Biology publications, USA.
8. Watson J.D. 2009, A Passion for DNA: Genes, Genomes & Society, Cold Spring Harbor Laboratory press (CSHL).

**Instruction hours/week: L: 4 T: 0 P: 0 Marks:100****End Semester Exam: 3 Hours****Course Objectives****Equip the students with:**

- Methods to identify plants/herbs with a specific biological activity
- Evaluating the presence/absence of flavonoids, terpenoids, glycosides and steroids
- Analysis of the crude extract in vitro to evaluate its free radical scavenging activity
- Fractionation methods to isolate a specific compound and its evaluation
- Enhancing the production of secondary metabolite using plant tissue culture
- Assessment of the efficacy of a specific plant metabolite using animal cell culture

**Course Outcomes:****After successful completion, the students will understand:**

1. The availability of traditional knowledge search resources
2. Identify flavonoids, terpenoids, glycosides and steroids
3. Analyze the crude extract in vitro for its free radical scavenging activity
4. Column chromatography, TLC
5. How to enhance the production of secondary metabolite using plant tissue culture
6. Animal cell culture

**UNIT I: Medicinal plants**

Medicinal plants-bioactive principles in medicinal plants methods of extraction, isolation, separation and screening, pharmacologically active plants-CNS, CVD, Hypoglycemic, Hepatoprotective, anti allergic, anticancer, immunoactive plants, plants protecting against oxidative stress, chemotherapeutic products.

**UNIT II: Free radicals**

Free radicals –types, sources, importance, production, free radicals induced damages, lipid peroxidation, measurement of free radicals, disease caused by radicals, reactive oxygen species, antioxidant defence system, enzymic and non-enzymic antioxidants, role of antioxidants in prevention of diseases, phytochemicals as antioxidants.

**UNIT III: Metabolites of Plant**

Alkaloids, flavonoids, terpenoids, phenols-Occurrence, distribution & functions, Production of secondary metabolite in plants, stages of secondary metabolite production, uses of tissue culture techniques, elicitation, biotransformation- production of pharmaceutical compounds.

**UNIT IV: Plant cell culture**

Principles-callus, meristem and organ culture, culture methods, culture media & preparations, plant regeneration, protoplast technology, micropropagation in plants, somatic embryogenesis, somoclonal selection.

#### **UNIT V: Animal cell culture**

Animal cell culture: Culture media, Serum and protein free defined media and their application. Functions of different constituents of culture medium. Role of carbon dioxide, growth factors, glutamine in cell culture. Cell lines, primary culture and culture maintenance.

Experimental animals and Animal handling - Sacrification, collection of sample. Ethical issues for animal handling.

#### **SUGGESTED READING**

1. Dubey R.C. (2009). Text book of Biotechnology, S. Chand & Company Ltd. New Delhi.
2. Freshney, R. I., & Freshney, M. G. (2010). In Freshney, R. I. (ed.), Animal cell culture: a practical approach, 2nd ed. IRL Press at Oxford University Press. USA.
3. Jain V. K. (2010). Fundamentals of plant physiology, C. Chand and Company Ltd, New Delhi.
4. Purohit. S.S. (2005). Agricultural Biotechnology, Dr. Updesh Purohit Publishers, Jodhpur. India
5. Singh. M.P and Panda. H (2005). Medicinal Herbs with their formulations, Daya Publishing House, New Delhi.

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**Instruction hours/week: L: 4 T: 0 P: 0 Marks:100****End Semester Exam: 3 Hours****Course Objectives****Equip the students with:**

- Methods to identify plants/herbs with a specific biological activity
- Evaluating the presence/absence of flavonoids, terpenoids, glycosides and steroids
- Analysis of the crude extract in vitro to evaluate its free radical scavenging activity
- Fractionation methods to isolate a specific compound and its evaluation
- Enhancing the production of secondary metabolite using plant tissue culture
- Assessment of the efficacy of a specific plant metabolite using animal cell culture

**Course Outcomes:****After successful completion, the students will understand:**

7. The availability of traditional knowledge search resources
8. Identify flavonoids, terpenoids, glycosides and steroids
9. Analyze the crude extract in vitro for its free radical scavenging activity
10. Column chromatography, TLC
11. How to enhance the production of secondary metabolite using plant tissue culture
12. Animal cell culture

**UNIT I: Clinical Enzymology**

Clinical significance of Phosphatases, transaminases, 5'-nucleotidase, Gamma -glutamyl transferase, Lactate Dehydrogenase, Creatine Phosphokinase.

Diagnostic enzymes in hepatobiliary disease, Atherosclerosis, Myocardial infarction, renal dysfunction. Cancer markers for oral, prostate, colorectal breast and GI tract cancer, oncofetal cancer markers.

**UNIT II: Free radicals**

Formation of free radicals, autoxidation initiated by oxygen radicals, Influence of free radicals in metal toxicity. Free radicals and cancer. Oxidative process in tissue injury. Detection of free radicals and radical ions. Role of free radicals in diseases.

**UNIT III: Antioxidants**

**Enzymic antioxidants-** Chemistry, mechanism, antioxidant effect of SOD, catalase, Glutathione Peroxidase.

**Non Enzymic antioxidants-** source, chemistry, toxicity, biochemical functions, bioavailability, bioassays, Antioxidant effects of Vit A, Vit C, Vit E, glutathione and selenium.

**Trace elements** - Introduction, sources, biochemical functions of zinc, copper and magnesium and iron.

#### **UNIT IV: Medicinal plants**

Medicinal plants-bioactive principles in medicinal plants methods of extraction, isolation, separation and screening ,Pharmacologically active plants-CNS,CVS, Hypoglycemic, Hepatoprotective ,anti allergic ,anticancer, immunoactive plants, plants protecting against oxidative stress, chemotherapeutic products.

#### **UNIT V: Toxicity**

Effects of physiochemical and biological factors on heavy metal toxicity, toxic mechanism-Carcinogenesis, teratogenesis and immunotoxicity. Bioassays for heavy metal toxicity, pathological, histopathological examinations for heavy metal toxicity.

#### **SUGGESTED READING**

1. Chatterjee M.N. and Rana Sinde, (2006) Text Book of Medical Biochemistry, 6<sup>th</sup> Edition, Jaypee Brothers, Medical Publishers, New Delhi.
2. Harper's Illustrated Biochemistry (2009) 28<sup>th</sup> Edition McGraw Hill, Mumbai.
3. Nelson and Cox (2005). Principles of Biochemistry by, 4<sup>th</sup> Edition, Mumbai.
4. Devlin (2006). Biochemistry with Clinical Correlation, 6<sup>th</sup> Edition, John Wiley & Sons, USA.
5. Ramnik Sood (2009). Medical Laboratory Technology,; Jaypee Brothers Medical Publishers, New Delhi
6. Tietz. Fundamentals of Clinical Chemistry (2008). 6<sup>th</sup> Edition, Elsevier, USA.
7. Voet D.and Voet J (2008) .Biochemistry, 3<sup>rd</sup> Edition, J. Wiley & Sons, USA.

**Instruction hours/week: L: 4 T: 0 P: 0 Marks:100****End Semester Exam: 3 Hours****Course Objectives:**

Equip the students with

- The organization of plant genome
- Molecular markers of plant tissues
- Growth regulators of plant
- Cloning strategies
- Transformation methods
- Manipulation of plant nucleic acids

**Course Outcomes:**

After successful completion, the students will understand:

1. Organization of plant genome
2. Molecular markers of plant tissues
3. Growth regulators of plant
4. Cloning strategies
5. Transformation methods
6. Manipulation of plant nucleic acids

**UNIT I: Plant genome**

Plant genome organization, structural features of a representative plant gene. Organization of chloroplast genome and mitochondrial genome. Molecular markers (AFLP, ISSR and RAPD). Plant tissue culture media, plant hormones and growth regulators in tissue culture, preparation of suitable explants. Micropropagation of plants - somatic embryogenesis, protoplast culture, somatic hybridization and synthetic seeds.

**UNIT II: Cloning strategies**

Tools for cutting and joining of DNA; gene transfer techniques; Methods of selection and screening of recombinant DNA. Construction of genomic libraries and cDNA libraries - probe construction and labelling (radio and non-radio). Molecular mechanism of anti-sense technology - inhibition of splicing, disruption of RNA structure & capping - application of anti-sensing technology.

**UNIT III: Gene regulation**

Inducible enzymes, regulatory mutations, repressor, operon, promoter, catabolic repression, repressible enzyme systems, control by attenuation, positive control, gene regulation in eukaryotes, transcriptional regulation, post transcriptional regulation, hormones & gene expression; viruses & gene expression, genetic control of pattern formation in plant development.

#### **UNIT IV: Plant transformation technology**

Symbiotic nitrogen fixation in legumes by rhizobia - biochemistry and molecular biology. Binary vectors, Use of 35s & other promoters genetic markers methods of nuclear transformation viral vectors & their applications, Use of reporter gene, Particle bombardment, Electroporation, Microinjection, Chloroplast transformation, Transformation of monocots, Transgene stability & gene silencing in Plant transformation.

#### **UNIT V: Plant manipulation and its applications**

Transgenic plants - for– biotic (weeds, insects, viruses, fungi and bacteria) and abiotic (drought, salt, temperature, poor soil quality and oxidative) stress tolerance. Production of secondary metabolites production. Molecular farming (improvement in protein, lipids, carbohydrates. Plant antibodies, vaccines, therapeutic proteins and active principles. Biofortification of important crops (rice and banana).

#### **SUGGESTED READING**

1. Altman A, Hasegawa P M. 2012 “Plant Biotechnology and agriculture. Prospect for the 21<sup>st</sup> century” Academic Press, USA.
2. Brown T. A. 2010. Gene Cloning and DNA Analysis: an introduction, 6<sup>th</sup> edition, Wiley-Blackwell Publisher, UK.
3. Chawla H.C. 2009 Introduction to Plant Biotechnology 3<sup>rd</sup> Edition, Oxford & IBH publication Pvt .Ltd, New Delhi.
4. Davies K. 2004. Plant Pigments and their Manipulation – Annual plant reviews, vol 14 Blackwell Publication, UK.
5. Glick and Paster mark, 2002. Molecular Biotechnology - Principles and Applications in Recombinant DNA. Panima Publishing Co-operation, Bangalore.
6. Primrose S.B and R.M. Twyman. 2003. Principles of Genome Analysis. Blackwell Publishing, Oxford.
7. Slater A, Scott NW, Fowler MR. 2008 Plant Biotechnology: the genetic manipulation of plants, Oxford Press, UK.
8. Winnacker E. 2003. From Gene to Clones; Introduction to gene technology, 4<sup>th</sup> edition, Panima Publisher, India.



**Course Objectives:**

- Equip the students with:
- Applications and limitations of cell based studies
  - Aseptic methods to perform animal cell culture
  - Types of cell culture techniques
  - Assessment of cell proliferation
  - Assessment of cell differentiation
  - Scale up technologies

**Course Outcomes:**

After successful completion, the students will understand:

1. The need and circumstances for the cell based studies
2. Sterile working culture
3. Suspension and adherent cell culture
4. Thymidine incorporation, WST-1 Assays
5. Galactosidase staining, von-kossa, and alizarin red staining
6. Organ culture

**UNIT I: Introduction to animal cell culture**

Introduction, importance, history of cell culture development, different tissue culture techniques including primary and secondary culture, continuous cell lines, suspension culture, organ culture, advantages and limitations medical/pharmaceutical products of animal cell culture-genetic engineering of animal cells and their applications. Risks in a tissue culture laboratory and safety - biohazards.

**UNIT II: types of cell culture**

Different types of cell culture media, growth supplements, serum free media, balanced salt solution, other cell culture reagents, culture of different tissues and its application. Facilities for animal cell culture-infrastructure, equipment, culture vessels. Biology and characterization of cultured cells-cell adhesion, proliferation, differentiation, morphology of cells and identification.

**UNIT III: Types of Techniques**

Primary cell culture techniques - mechanical disaggregation, enzymatic disaggregation, separation of viable and non-viable cells. Mass culture of cells - manipulation of cell line selection - types of cell lines -maintenance of cell lines - immobilization of cells and its

application - synchronization of cell cultures and cell division - production of secondary metabolites - biotransformation - Induction of cell line mutants and mutations - cryopreservation – germplasm conservation and establishment of gene banks.

#### **UNIT IV: Animal cell culture scale up**

Animal cell culture scale up: Scale up in suspension - stirrer culture, continuous flow culture, air-lift fermentor culture; Scale up in monolayer - Roller bottle culture, multi surface culture, multi array disks, spirals and tubes - monitoring of cell growth. Organ culture - whole embryo culture - specialized culture techniques - measurement of cell death.

#### **UNIT V: Tissue engineering**

Tissue engineering: Design and engineering of tissues - tissue modeling. Embryonic stem cell engineering - ES cell culture to produce differential cells - Human embryonic stem cell research. Transgenic animals-transgenic animals in xenotransplantation.

#### **SUGGESTED READING**

1. Butler. M. 2004. Animal Cell Culture and Technology, BIOS Scientific Publishers, Taylor and Francis Group. U. K.
2. Freshney, R. I., & Freshney, M. G. 2010. In Freshney, R. I. (ed.), Animal cell culture: a practical approach, 2nd ed. IRL Press at Oxford University Press.
3. Gupta P.K. (2010), Biotechnology & Genomics, 5th Reprint, Rastogi Publications Meerut.
4. Ranga M.M., Animal Biotechnology, (2007) Agrobios, India.
5. Satyanarayana, U., 2006. Biotechnology Books and Allied (P) Ltd. India.