

FORM – II A

**APPLICATION FOR ENVIRONMENTAL APPROVAL OF CLINICAL TRIALS/
PHARMACEUTICALS / VETERINARY DRUGS DERIVED FROM LIVING
MODIFIED ORGANISMS / HAZARDOUS MICRO-ORGANISMS.**

Part A

- (a) Not all the points included will apply to every case. It is to be expected, therefore, that individual applicant will address only the particular parameters that are appropriate to individual situations. In each case where it is not technically possible or it does not appear necessary to give the information, the reasons shall be stated.
- (b) The details required in response to each parameter are also likely to vary according to the nature and scale of the proposed release.
- (c) The description of the methods used or the reference to the standardized or internationally recognized methods shall also be mentioned in the form together with the name of the body or bodies responsible for carrying out the studies.
- (d) A 1-2 page summary of the proposal shall be appended with application.

Part B

- 1. Name of the Applicant
- 2. Name of organization/firm
- 3. Approval required
 - (i) Manufacture
 - (ii) Import
 - (iii) Marketing
- 4. Quantity per year of the product to be manufactured/imported/marketed.

Part C

- 1. Name of the product:
 - (i) Human medicine
 - (ii) Veterinary medicine
 - (iii) Food product
 - (iv) Others (please specify)
- 2. Intended use (Pathological, Metabolic and Immune Response)
- 3. Safety Concern:

- (i) Donor (heterologous Nucleic acid segments from sources)
- (ii) Vector (DNA) molecules to which heterologous nucleic acid segments are joined for transfer to hosts)
- (iii) Hosts (living cells or organisms into which rDNA molecules are introduced)

4. Production method (the production method should give the details of the cell lines used, the information on the production of the recombinant proteins within or outside the cells, the concentration of the products in units per litre of the fermented broth as well as the concentration in physical weights. The description should also include in brief the methods applied for reducing the genomic particles, proteins and living contaminants including viruses, bacteria, fungi, parasites. Etc.)

(a) Characterisation of the system of production used

(i) Details of the expression system:

- Description of the host cell line
- Identification of the genera and the species.
- The risks involved in handling the cell line.
- The classification of the cell line according to the Government of India's Biosafety guidelines or any other accepted Recombinant cell line usage guideline.
- The method(s) of maintenance and growth of the cell line.
- The nature and hazards of using substrates, inducing agents, etc.

(ii) Characteristics of the target gene and vector

- The full description of the source of the target gene.
- The composition of the vector used indicating the promoter sequence as well as the regulatory mechanisms utilized in the expression cassette.
- Schematic diagrams of the expression cassette to describe fully the marker genes used.
- The restriction sites related to specific endo nucleases, and the cell lines used for shuttling and amplification of the expression cassette.
- The method of constructing the target gene along with all the sequences added or deleted.
- The extent of target gene amplification into the host genome.
- The target gene should also contain, along with the nucleotide sequence, the description of the amino acids below the codons.

(iii) Approaches adopted for expression of the gene.

- The description of the transcribed messenger RNA with its analysis of sequence and identification procedure.

- The translation information indicating whether the protein product is Chimaric, whether the expression is found as inclusion bodies or as intracellular protein or whether the protein is secreted out.
- The extent of the target protein produced as the percentage of the total cell dry mass as well as its percentage compared to the total proteins of the cell.
- The quantity of the target protein produced after the cell growth per litre of the fermented broth.
- The full sequence of the recombinant gene alongwith the promoters the marker genes and the terminator se sequence.

(b) Description of the production process

(i) Production set up

- The handling of the stock cell lines.
- The evaluation and uses of the cell lines in pre-fermentor processes.
- The preparation of the seed vessel.
- The main production fermentation conditions need to be described in brief.

(ii) Growth Kinetics

- Graphical plots of the versus substrate usage, optical density change, biomass formation, protein products formation, inducing agents used and their effects on the target protein formation.
- The effects of change of standard parameters like pH dissolved oxygen, temperature etc. in the main production fermentor.

(iii) Fermentation parameters

- The pH, temperature, aeration, and rpm of the shaft.
- Volume of seed to the volume of main fermentor.
- Main substrates used inducing agents used if any.
- The fermentation time and the concentration of the target protein in the fermented broth.

(c) In process Control Methods

- All the analytical methods used for this in-process control must be described to ensure the regulatory authorities that the chances of entry of unwanted products have been minimized.

(d) Description of the raw and processing materials used

- Description of all the raw materials and processing materials used starting from the stock cells handling to the finished dosage form must be in a tabular form.
- Grades of materials used and their usual sourcing.

- (e) Description of the plant and Machinery used
- List of all the equipment alongwith the indicative capacity.
 - Names of the manufacturers.
 - List of main production equipment and the quality control equipment.

- (f) Description of the building and facilities created for the manufacture

- The manufacturing area.
- The flow of the process/operation starting from the stock culture area to the finishing area.
- The plot plan of various floors used in the manufacture and processing.
- The building diagram in the plan and/or elevation in the outline indicating the cleanliness maintained in different sections such as class 10,000, class 1000 and class 100 areas etc.
- Description of the air handling system which is very important.
- A separate write-up indicating the movement of operating personnel in the area.

5. Approaches for the extraction and purification of the product. The purification methods should particularly indicate the modes of elimination of viruses, bacteria, fungi, parasites, etc., if the cell lines are mammalian origin. It should also include methods for the removal of genomic particles, and proteins irrespective of cell lines/hosts used for production, removal of adjuvants, reagents chemicals including vectors, donor and recipient organisms foreign DNA and adventitious materials associated with production methods.

- The methods of handling the cells.
- The methods of isolating the cell soup containing the target protein the methods of enzyme treatment, if any.
- The methods of concentration, precipitation (if applied), reconstitution, salt separation (if applicable), absorption and desorption methods, chromatographic methods used if any, ultracentrifugation methods if used.
- Sterilization and final dosage formulation.
- Processes to obtain the product in the finished dosage form.

6. Quality Control and Quality Assurance

- (a) Bulk material:

- Test relating to the identification of the stock cells.
- The plasmid construct retention in the cells during cell growth.
- The media used.

- The procedures adopted for testing and the percentage retention of the target plasmid.
 - Description of the contamination test of the stock culture to assess and monitor the microbial contamination including the media used and the procedures adopted.
 - The criteria of acceptance of contamination free culture.
 - Characterisation of the bio-active molecule produced by monitoring physical & chemical properties of the molecules by the following or more authentic method should be provided.
- Microscopic examination including light, phase contract and electron microscopic studies.
 - UV Spectroscopic analysis to show the absorption spectra of the product and comparison of this with authentic material.
 - Density gradient centrifugation to show single peak.
 - Pattern in High performance liquid chromatography to indicate how many peaks or if only one peak is noticed in the produced and purified bulk.
 - Iso-electric focussing analysis to show the pl values.
 - Western Blot and SDS-PAGE to map the peptide/target protein.
 - Existence of special bonds like disulfide bonds by breaking the protein and subjecting to SDS-PAGE mapping.
 - Immunodiffusion Test to find out the absence of contaminants or the extent of the presence of contaminants.
 - Amino acid composition of the purified protein and its comparison with the authentic material.
 - N-Terminal Amino Acid Sequence analysis and its comparison with the gene construct used.
 - Determination of the biological activity in the animal model.
 - Determination of Contaminants per milligram or any convenient unit of the manufacturing bulk purified protein is also to be carried out to indicate the extent of contaminant nucleic acid stretches, proteins, carbohydrates, lipids, detergents, salts and other processing chemicals used in the purification. The impacts of the presence of these contaminants are also to be indicated with authentic references if any to ensure that the risks associated with their presence are minimal. The limits of contaminants and the acceptance criteria need to be quantified for each contaminant.

(b) Formulated Material:

- The ingredients incorporated subsequently in the manufacture. The specification of the final product.
- The Quality Control Department would have to certify that the final product has the results within the specifications prescribed and accepted by the Regulatory Authorities.
- The documentation should therefore indicate the following specifically:

(i) Product presentation.

- (ii) Physical appearance.
 - (iii) Product inserts, literature and label claim
 - (iv) Volume/Quantity per pack/dose.
 - (v) Potency of the product.
 - (vi) Particulate matter limits for liquid.
 - (vii) Preservative usage percentage.
 - (viii) PH
 - (ix) Other extraneous materials used their extent such as contents of DNA, RNA, Carbohydrates, Lipids, processing materials like detergents, salts, etc.
 - (x) Protein content in the product.
 - (xi) Sterility status
 - (xii) Toxicity information
 - (xiii) Pyrogen status.
7. Possible Hazards to environment from release of GMO/nucleic acid/micro-organisms or products.
8. Clinical field trials done in India/abroad (whether permission of Institutional Ethics Committee obtained)
9. Stability and Shelf Life of product.
10. Method of disposal of vials of syringes/wastes matter.
11. Regulatory status in India/abroad (enclosed certificates like free sale certificate/GMP certificate/certificates granted by Health & Environment Authorities of the country of origins signed by applicant. In case the certificate is issued by the concerned authority of country of origin, the certificate should be endorsed/ authenticated by Indian Embassy/High Commission/Consulate in that country.
- Every certificate shall be accompanied by other statutory information like manufacturing batch, no. date of manufacture, date of analysis, date of release of the certificate, signatory to the certificate etc.
 - The final formulated product needs to be certified for acceptance by the manufacturer.

Applicant's signature with seal.