In addition, the label on the outer package states:
- the route of administration,
- the period of validity or the expiry date,
- the name and concentration of any added antimicrobial preservative,
- where applicable, any special storage conditions.

01/2008:0784

RECOMBINANT DNA TECHNOLOGY, PRODUCTS OF

Producta ab arte ADN recombinandorum

This monograph provides general requirements for the development and manufacture of products of recombinant DNA technology. These requirements are not necessarily comprehensive in a given case and requirements complementary or additional to those prescribed in this monograph may be imposed in an individual monograph or by the competent authority.

The monograph is not applicable to modified live organisms that are intended to be used directly in man and animals, for example as live vaccines.

DEFINITION

Products of rDNA technology are produced by genetic modification in which DNA coding for the required product is introduced, usually by means of a plasmid or a viral vector, into a suitable micro-organism or cell line, in which that DNA is expressed and translated into protein. The desired product is then recovered by extraction and purification. The cell or micro-organism before harbouring the vector is referred to as the host cell, and the stable association of the two used in the manufacturing process is referred to as the host-vector system.

PRODUCTION

Production is based on a validated seed-lot system using a host-vector combination that has been shown to be suitable to the satisfaction of the competent authority. The seed-lot system uses a master cell bank and a working cell bank derived from the master seed lot of the host-vector combination. A detailed description of cultivation, extraction and purification steps and a definition of the production batch shall be established.

Where products of rDNA technology are manufactured using materials of human or animal origin, the requirements of chapter *5.1.7. Viral safety* apply.

The determination of the suitability of the host-vector combination and the validation of the seed-lot system include the following elements.

CLONING AND EXPRESSION

The suitability of the host-vector system, particularly as regards microbiological purity, is demonstrated by:

Characterisation of the host cell, including source, phenotype and genotype, and of the cell-culture media;

Documentation of the strategy for the cloning of the gene and characterisation of the recombinant vector, including:

i. the origin and characterisation of the gene;

ii. nucleotide-sequence analysis of the cloned gene and the flanking control regions of the expression vector; the cloned sequences are kept to a minimum and all relevant expressed sequences are clearly identified and confirmed at the RNA level; the DNA sequence of the cloned gene is normally confirmed at the seed-lot stage, up to and beyond the normal level of population doubling for full-scale fermentation; in certain systems, for example, where multiple copies of the gene are inserted into the genome of a continuous cell line, it may be inappropriate to sequence the cloned gene at the production level; under these circumstances, Southern blot analysis of total cellular DNA or sequence analysis of the messenger RNA (mRNA) may be helpful, particular attention being paid to the characterisation of the expressed protein;

iii. the construction, genetics and structure of the complete expression vector;

Characterisation of the host-vector system, including:

i. mechanism of transfer of the vector into the host cells;

ii. copy number, physical state and stability of the vector inside the host cell;

iii. measures used to promote and control the expression.

CELL-BANK SYSTEM

The master cell bank is a homogeneous suspension of the original cells already transformed by the expression vector containing the desired gene, distributed in equal volumes into individual containers for storage (for example, in liquid nitrogen). In some cases it may be necessary to establish separate master cell banks for the expression vector and the host cells.

The working cell bank is a homogeneous suspension of the cell material derived from the master cell bank(s) at a finite passage level, distributed in equal volumes into individual containers for storage (for example, in liquid nitrogen).

In both cell banks, all containers are treated identically during storage and, once removed from storage, the containers are not returned to the cell stock.

The cell bank may be used for production at a finite passage level or for continuous-culture production.

Production at a finite passage level

This cultivation method is defined by a limited number of passages or population doublings which must not be exceeded during production. The maximum number of cell doublings, or passage levels, during which the manufacturing process routinely meets the criteria described below must be stated.

Continuous-culture production

By this cultivation method the number of passages or population doublings is not restricted from the beginning of production. Criteria for the harvesting as well as for the termination of production have to be defined by the manufacturer. Monitoring is necessary throughout the life of the culture; the required frequency and type of monitoring will depend on the nature of the production system and the product.

Information is required on the molecular integrity of the gene being expressed and on the phenotypic and genotypic characteristics of the host cell after long-term cultivation. The acceptance of harvests for further processing must be clearly linked to the schedule of monitoring applied and a clear definition of a 'batch' of product for further processing is required.

VALIDATION OF THE CELL BANKS

Validation of the cell banks includes:

i. stability by measuring viability and the retention of the vector;

ii. identity of the cells by phenotypic features;

iii. where appropriate, evidence that the cell banks are free from potentially oncogenic or infective adventitious agents (viral, bacterial, fungal or mycoplasmal); special attention

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has to be given to viruses that can commonly contaminate the species from which the cell line has been derived; certain cell lines contain endogenous viruses, for example, retroviruses, which may not readily be eliminated; the expression of these organisms, under a variety of conditions known to cause their induction, shall be tested for;

iv. for mammalian cells, details of the tumorigenic potential of the cell bank shall be obtained.

CONTROL OF THE CELLS

The origin, form, storage, use and stability at the anticipated rate of use must be documented in full for all cell banks under conditions of storage and recovery. New cell banks must be fully validated.

VALIDATION OF THE PRODUCTION PROCESS

Extraction and purification

The capacity of each step of the extraction and purification procedure to remove and/or inactivate contaminating substances derived from the host cell or culture medium, including, in particular, virus particles, proteins, nucleic acids and added substances, must be validated.

Validation studies are carried out to demonstrate that the production process routinely meets the following criteria:

- exclusion of extraneous agents from the product; studies including, for example, viruses with relevant physico-chemical features are undertaken, and a reduction capacity for such contaminants at each relevant stage of purification is established;
- adequate removal of vector, host-cell, culture medium and reagent-derived contaminants from the product; the reduction capacity for DNA is established by spiking; the reduction of proteins of animal origin can be determined by immunochemical methods;
- maintenance within stated limits of the yield of product from the culture;
- adequate stability of any intermediate of production and/or manufacturing when it is intended to use intermediate storage during the process.

Characterisation of the substance

The identity, purity, potency and stability of the final bulk product are established initially by carrying out a wide range of chemical, physical, immunochemical and biological tests. Prior to release, each batch of the product is tested by the manufacturer for identity and purity and an appropriate assay is carried out.

Production consistency

Suitable tests for demonstrating the consistency of the production and purification are performed. In particular, the tests include characterisation tests, in-process controls and final-product tests as exemplified below.

AMINO-ACID COMPOSITION

Partial amino-acid sequence analysis. The sequence data permit confirmation of the correct *N*-terminal processing and detection of loss of the *C*-terminal amino acids.

Peptide mapping. Peptide mapping using chemical and/or enzymatic cleavage of the protein product and analysis by a suitable method such as two-dimensional gel electrophoresis, capillary electrophoresis or liquid chromatography must show no significant difference between the test protein and the reference preparation. Peptide mapping can also be used to demonstrate correct disulphide bonding.

DETERMINATION OF MOLECULAR MASS

Cloned-gene retention. The minimum percentage of cells containing the vector or the cloned gene after cultivation is approved by the relevant authority.

Total protein. The yield of protein is determined.

Chemical purity. The purity of the protein product is analysed in comparison with a reference preparation by a suitable method such as liquid chromatography, capillary electrophoresis or sodium dodecyl sulphate polyacrylamide gel electrophoresis.

Host-cell-derived proteins. Host-cell-derived proteins are detected by immunochemical methods, using, for example, polyclonal antisera raised against protein components of the host-vector system used to manufacture the product, unless otherwise prescribed. The following types of procedure may be used: liquid-phase displacement assays (for example, radio-immunoassay), liquid-phase direct-binding assays and direct-binding assays using antigens immobilised on nitrocellulose (or similar) membranes (for example, dot-immunoblot assays, Western blots). General requirements for the validation of immunoassay procedures are given under *2.7.1. Immunochemical Methods.* In addition, immunoassay methods for host-cell contaminants meet the following criteria.

- *Antigen preparations.* Antisera are raised against a preparation of antigens derived from the host organism, into which has been inserted the vector used in the manufacturing process that lacks the specific gene coding for the product. This host cell is cultured, and proteins are extracted, using conditions identical to those used for culture and extraction in the manufacturing process. Partly purified preparations of antigens, using some of the purification steps in the manufacturing process, may also be used for the preparation of antisera.
- *Calibration and standardisation.* Quantitative data are obtained by comparison with dose-response curves obtained using standard preparations of host-derived protein antigens. Since these preparations are mixtures of poorly defined proteins, a standard preparation is prepared and calibrated by a suitable protein determination method. This preparation is stored in a stable state suitable for use over an extended period of time.
- *Antisera.* Antisera contain high-avidity antibodies recognising as many different proteins in the antigen mixture as possible, and do not cross-react with the product.

Host-cell- and vector-derived DNA. Residual DNA is detected by hybridisation analysis, using suitably sensitive, sequence-independent analytical techniques or other suitably sensitive analytical techniques.

Hybridisation analysis

DNA in the test sample is denatured to give single-stranded DNA, immobilised on a nitrocellulose or other suitable filter and hybridised with labelled DNA prepared from the host-vector manufacturing system (DNA probes). Although a wide variety of experimental approaches is available, hybridisation methods for measurement of host-vector DNA meet the following criteria.

- *DNA probes.* Purified DNA is obtained from the host-vector system grown under the same conditions as those used in the manufacturing process. Host chromosomal DNA and vector DNA may be separately prepared and used as probes.
- *Calibration and standardisation.* Quantitative data are obtained by comparison with responses obtained using standard preparations. Chromosomal DNA probes and vector DNA probes are used with chromosomal DNA and vector DNA standards, respectively.

Standard preparations are calibrated by spectroscopic measurements and stored in a state suitable for use over an extended period of time.

– *Hybridisation conditions.* The stringency of hybridisation conditions is such as to ensure specific hybridisation between probes and standard DNA preparations and the drug substances must not interfere with hybridisation at the concentrations used.

Sequence-independent techniques

Suitable procedures include: detection of sulphonated cytosine residues in single-stranded DNA (where DNA is immobilised on a filter and cytosines are derivatised *in situ*, before detection and quantitation using an antibody directed against the sulphonated group); detection of single-stranded DNA using a fragment of single-stranded DNA bound to a protein and an antibody of this protein. Neither procedure requires the use of specific host or vector DNA as an assay standard. However, the method used must be validated to ensure parallelism with the DNA standard used, linearity of response and non-interference of either the drug substance or excipients of the formulation at the dilutions used in the assay.

IDENTIFICATION, TESTS AND ASSAY

The requirements with which the final product (bulk material or dose form) must comply throughout its period of validity, as well as specific test methods, are stated in the individual monograph.

01/2008:2034

SUBSTANCES FOR PHARMACEUTICAL USE

Corpora ad usum pharmaceuticum

The statements in this monograph are intended to be read in conjunction with individual monographs on substances in the Pharmacopoeia. Application of the monograph to other substances may be decided by the competent authority.

DEFINITION

Substances for pharmaceutical use are any organic or inorganic substances that are used as active substances or excipients for the production of medicinal products for human or veterinary use. They may be obtained from natural sources or produced by extraction from raw materials, fermentation or synthesis.

This monograph does not apply to herbal drugs, herbal drug preparations or extracts, which are the subject of separate general monographs [*Herbal drugs (1433), Herbal drug preparations (1434), Extracts (0765)*].

Where medicinal products are manufactured using substances for pharmaceutical use of human or animal origin, the requirements of chapter *5.1.7. Viral safety* apply.

Substances for pharmaceutical use may be used as such or as starting materials for subsequent formulation to prepare medicinal products. Depending on the formulation, certain substances may be used either as active substances or as excipients. Solid substances may be compacted, coated, granulated, powdered to a certain fineness or processed in other ways. Processing with addition of excipients is permitted only where this is specifically stated in the Definition of the individual monograph.

Substance for pharmaceutical use of special grade. Unless otherwise indicated or restricted in the individual monographs, a substance for pharmaceutical use is intended for human and veterinary use, and is of appropriate quality for the manufacture of all dosage forms in which it can be used.

Polymorphism. Individual monographs do not usually specify crystalline or amorphous forms, unless bioavailability is affected. All forms of a substance for pharmaceutical use comply with the requirements of the monograph, unless otherwise indicated.

PRODUCTION

Substances for pharmaceutical use are manufactured by procedures that are designed to ensure a consistent quality and comply with the requirements of the individual monograph or approved specification.

The provisions of general chapter *5.10* apply to the control of impurities in substances for pharmaceutical use.

Whether or not it is specifically stated in the individual monograph that the substance for pharmaceutical use:

– is a recombinant protein or another substance obtained as a direct gene product based on genetic modification, where applicable, the substance also complies with the requirements of the general monograph on *Products of recombinant DNA technology (0784)*;
– is obtained from animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge, where applicable, the substance also complies with the requirements of the general monograph on *Products with risk of transmitting agents of animal spongiform encephalopathies (1483)*;
– is a substance derived from a fermentation process, whether or not the micro-organisms involved are modified by traditional procedures or recombinant DNA (rDNA) technology, where applicable, the substance complies with the requirements of the general monograph on *Products of fermentation (1468)*.

If solvents are used during production, they are of suitable quality. In addition, their toxicity and their residual level are taken into consideration (*5.4*). If water is used during production, it is of suitable quality.

If substances are produced or processed to yield a certain form or grade, that specific form or grade of the substance complies with the requirements of the monograph. Certain functionality-related tests may be described to control properties that may influence the suitability of the substance and subsequently the properties of dosage forms prepared from it.

Powdered substances may be processed to obtain a certain degree of fineness (*2.9.12*).

Compacted substances are processed to increase the particle size or to obtain particles of a specific form and/or to obtain a substance with a higher bulk density.

Coated active substances consist of particles of the active substance coated with one or more suitable excipients.

Granulated active substances are particles of a specified size and/or form produced from the active substance by granulation directly or with one or more suitable excipients.

If substances are processed with excipients, these excipients comply with the requirements of the relevant monograph or, where no such monograph exists, the approved specification.