2.3.S.2 Manufacture

The sites of manufacture, storage and quality control of the KN035 Drug Substance (DS) and cell banks are listed below (Table 2.2).

Table 2.2 Production Facilities for KN035 DS

Facilities	Function (s)
Suzhou Alphamab Co., Ltd. Building C23 BioBay Xinghu Street #218 Suzhou, Jiangsu, P.R. China	Manufacturing of bulk drug substance, in process testing, release and stability testing of DS and DP
BioReliance Todd campus West of Scotland science park Glasgow G20 OXA, UK; and BioReliance， Glencorse Building， Pentland Science Park Bush Loan, Penicuik EH26 0PZ, United Kingdom	Testing of MCB (other than the Porcine virus), WCB and unprocessed Bulk of KN035
BioReliance Todd campus West of Scotland science park Glasgow G20 OXA, UK	Testing of CHOK1S parental cell line WCB for porcine virus

The manufacturing of KN035 DS includes upstream and downstream processes. Upstream process includes steps from thawing one vial of working cell bank (WCB), cell cultivation and production bioreactor to generate unprocessed bulk. The downstream process includes harvest bulk clarification through depth filtration, and serial purification and impurity removals to generate the bulk drug substance. The flowchart of manufacturing process of KN035 DS is shown in Figure 2.1. No animal sourced materials were used in the manufacturing process of KN035 DS.

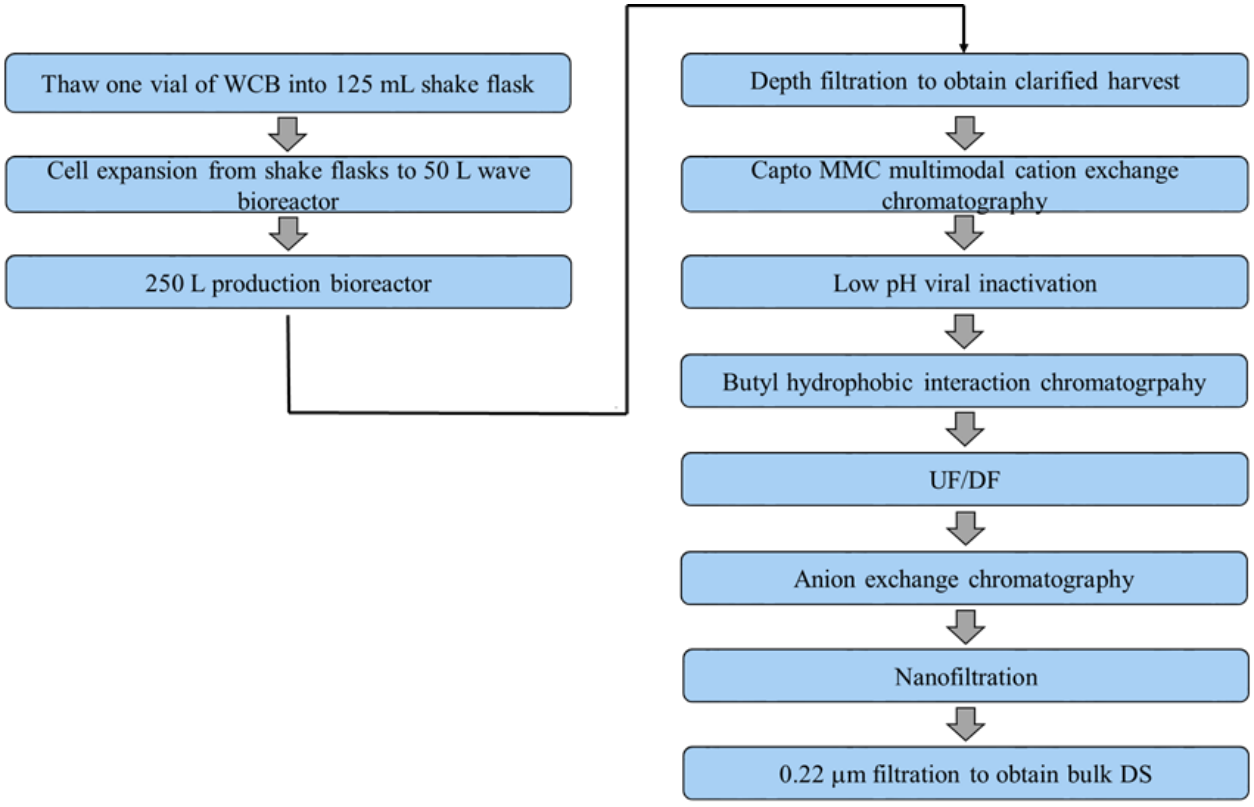

Figure 2.1 Manufacturing flowchart of KN035 drug substance.

The upstream manufacturing process includes expansion from one vial of WCB through shake flasks and the wave bag to the 250 L production bioreactor. The flowchart for the 250L scale upstream process including the process control is presented in Figure 2.2.

Upstream Process **In-Process Controls (IPC)**

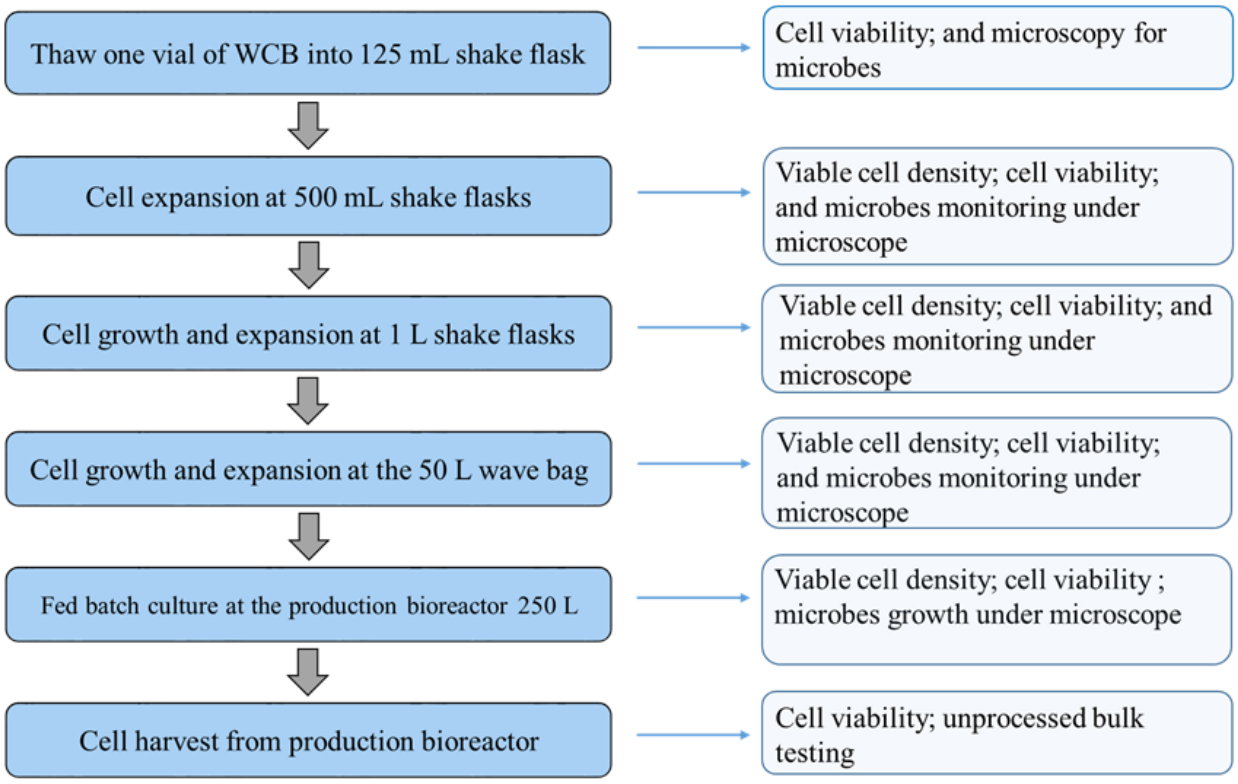

Figure 2.2 Upstream manufacturing process of KN035 DS

Cells are grown under the selection pressure of 25 μM MSX with 1 μMMTX in CD CHO medium only during the expansion in the shake flasks. No MSX and/or MTX is used during the cell expansion in the wave bag and in the production bioreactor. The unprocessed bulk testing results of the clinical batch 151202DS is presented in section 3.2.A.2. The unprocessed bulk is tested to be free of microbial contamination, mycoplasma, adventitious viruses, and MVM.

The downstream manufacturing process with in process controls are presented in Figure 2.3.

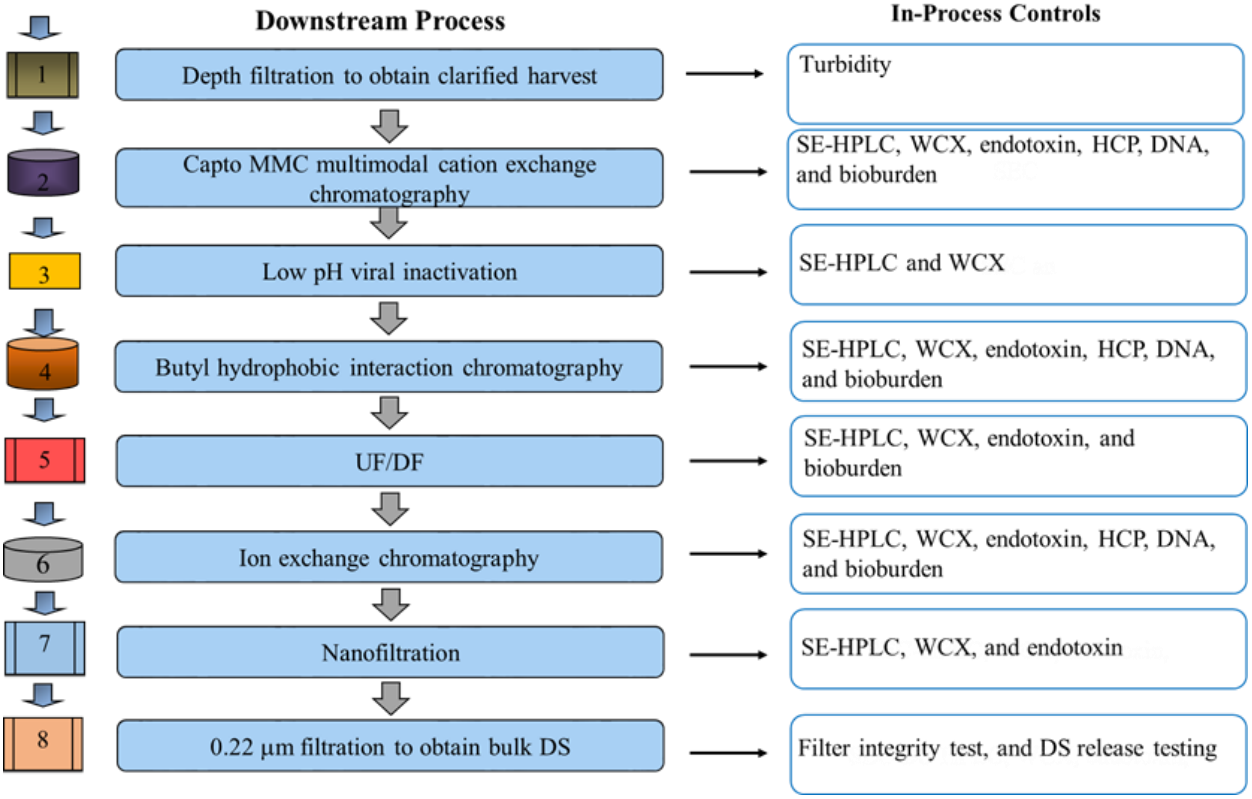

Figure 2.3 Downstream process and in-process controls for KN035 drug substance.

KN035 is expressed using recombinant deoxyribonucleic acid (DNA) technology and CHO expression system. The expression vector is shown in Figure 2.4.

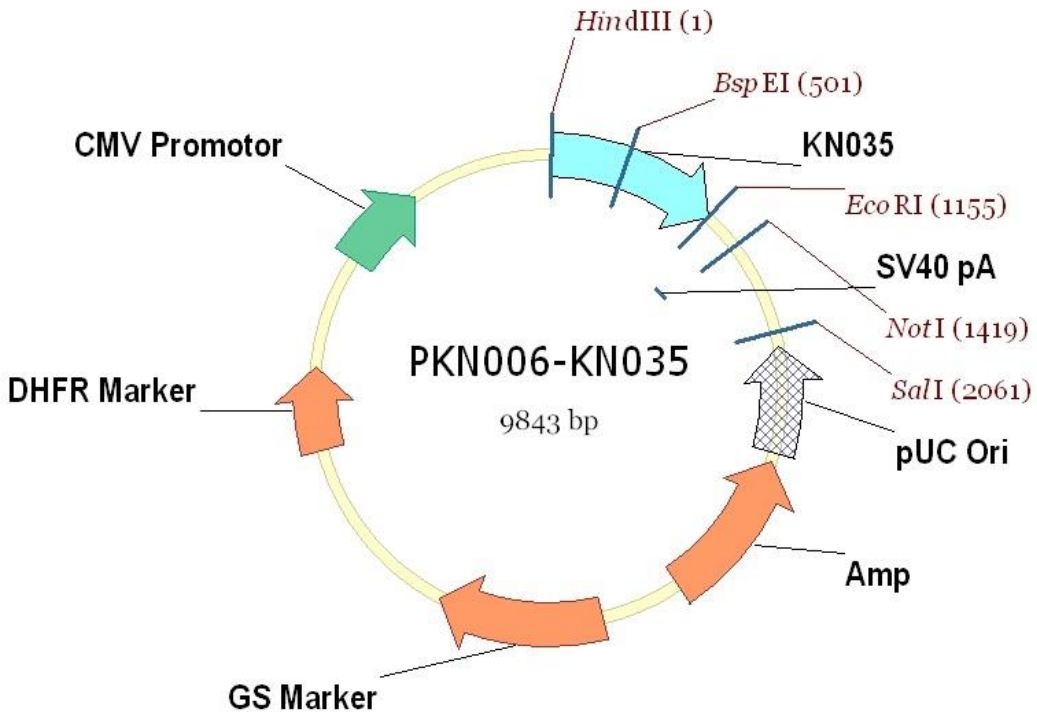

Figure 2.4 The plasmid map of pKN006- hu56VHH2-Ldel-FcAG (also named PKN006-KN035).

The host cell line CHOK1S was adapted from CHOK1 cell to grown in serum free medium. The MCB (lot 20100919) and WCB (lot 20101026) of the host cell line were established. The animal sourced materials used during the host cell line adaptation is presented in Table 2.3. No animal source materials were used for the WCB of CHOK1S establishment.

Table 2.3 Animal sourced materials used in the host cell line CHOK1S establishment.

Animal originated materials	Source	Tests performed by Vendor in CoA
FBS (Hyclone, catlog # SV30087, lot NUK0201)	South America	Free of mycoplasma and BSE; sterile.
FBS (Hyclone, catlog # SH30406.02, lot DPH0176)	New Zealand	Free of mycoplasma and BSE; sterile; Bovine viruses not detected (per 9 CFR 113.53)
Trypsin (Gibco, catlog # 25200, lot # 774748)	n/a	Free of porcine parvovirus and mycoplasma; and sterile.

The CoAs of the FBS and trypsin are attached at 3.2A.4.1 and 3.2.A.4.2.

The MCB and WCB were characterized and tested for safety. The testing results are presented in

Tables 2.4 and 2.5.

Table 2.4 Master Cell Bank Test Results

Tests	Methods	Acceptance Criteria	Results
Cell Line Identification	DNA fingerprinting	CHO cell line origin	CHO cell line origin
Sterility	USP/EP direct inoculation	No growth	No growth
Mycoplasma	EP/USP and FDA PTC 1993[1]: tests for the presence of agar-cultivable and no agar-cultivable mycoplasma	Not detected	Not detected
In Vitro Assay for adventitious viral contaminants (28-day assay)	MRC-5, Vero and CHOk1 Cells	Not detected	Not detected
In Vivo Assay for adventitious viral contaminants	Adult mice, suckling mice, guinea pigs &embryonated eggs	Not detected	Not detected
Detection of MAP	MAP test with LCMV challenge	Not detected	Not detected
Detection of HAP	HAP test with LCMV challenge	Not detected	Not detected
Retroviruses	Quantification of reverse transcriptase activity by ultracentrifugation and quantitative fluorescent product enhanced reverse transcriptase (QFPERT) assay.	Report results	Negative for the presence of retroviral RT activity
	Transmission electron microscopic examination (TEM)	Report results	Introcytoplasmic A-type particles were found.
	Extended S+ L- Assay: in vitro detection of xenotropic retrovirus by mink S+ L- focus assay	Report results	No foci observed and xenotropic virus was not detected.
	Detection of ecotropic murine retroviruses by extended XC plaque assay including TA effect	Not detected	Not detected
Bovine Viruses	Detection of viral contaminants according to CPMP & US 9 CFR requirements, also meets EP requirements.	Report results	No cytopathic effect; no cytological staining; no haemadsorption and no specific immunofluorescence.
	Real time PCR detection of bovine polyomavirus (BPyV)	Negative	Negative

1. FDA point to consider on the characterization of cell lines used to product biologicals (1993)

Table 2.5 Working Cell Bank Test Results

Tests	Methods	Acceptance Criteria	Results
Cell Line Identification	DNA fingerprinting	CHO cell line origin	CHO cell line origin
Sterility	USP/EP direct inoculation	No growth	No growth
Mycoplasma	EP/USP and FDA PTC 1993[1]: tests for the presence of agar-cultivable and no agar-cultivable mycoplasma	Not detected	Not detected
In Vitro Assay for adventitious viral contaminants (28-day assay)	MRC-5, Vero and CHOk1 Cells	Not detected	Not detected

1. FDA point to consider on the characterization of cell lines used to product biologicals (1993)