Validation Guide

LeCouple® KY Sampling Kit

Shanghai LePure Biotech Co., Ltd.

Catalogue

1. Overview

LeCouple® KY Sampling Kit used for aseptic sampling in biological product processes, and the safety and reliability of the product is extremely important. LeCouple® KY Sampling Kit, newly developed by Shanghai LePure Biotech Co., Ltd (LePure Biotech) has good performance in biological safety, chemical compatibility and physical properties. Through quality testing and verification, it provides a safe and reliable disposable product for users of biopharmaceutical enterprises.

This Validation Guide describes the physical and chemical characteristics, key control, quality standards, key quality parameters and other related information of the disposable sampling kit of LePure Biotech, so as to provide the basic information for the pre-use evaluation and testing of the product for customers.

1.1 Supply chain safety and security.

As a key consumable for biological products, suppliers of single-use systems need to provide comprehensive solutions to ensure the safety and controllability of their supply chains and avoid instability caused by extreme conditions.

LeCouple® Kit Sampling kit provides stable supply chain security by:

- localized production

The main production site is located in Shanghai, China, LePure Biotech is responsible for production, and controls the entire production process and film quality.

- multi-center production

In order to avoid the risks caused by a single production site, LePure Biotech is verifying the second production site overseas and will complete the second production site globally in 2023 to achieve the purpose of multi-center production.

1.2 Manufacturing site

LeCouple® KY Sampling Kits were manufactured in a Class C clean room, and dynamic environmental monitoring was carried out continuously during production. The test data showed that the air environment around the equipment could meet dynamic Class C standards.

The current production site of LeCouple® Sampling Kit manufactured by LeKrius film is located in Zhengbo Road, Lingang Free Trade Zone, Shanghai. The basic information is as follows:

The total area is 10078m^2, the Class D is 450m^2, the Class C is 3400m^2, and the Class C+A is 600m^2.

2 . Quality assurance

2.1 Quality management system

LePure follows ISO 9001: 2015 and ISO 13485:2016 to develop a quality management system, and has passed the ISO9001:2015 and ISO13485: 2016 system certification. The manufacture of LeCouple® KY Sampling Kits follows strict performance and industry safety standards. Critical production is conducted in a Class C clean room environment and is continuously monitored. Clean room personnel management, material management, process specification, equipment management and verification are all in accordance with good Manufacturing practice (GMP) requirements, to ensure that the standards and requirements in line with regulations.

2.2 Production management

LeCouple® KY Sampling Kits are produced in a Class C clean room.

The production equipment and instruments have passed perfect verification, and the calibration is completed before they are put into production. Through regular maintenance, the good condition of production equipment is guaranteed.

Production plans are managed through a unified internal process, take orders, drawings, customer requirements, and production schedules are all confirmed by relevant personnels.

The production materials are inspected before entering the warehouse, and they are released for the production of one-time products after meeting the requirements.

During the production of single-use products, check and confirm the production process and materials, including but not limited to: consistency with technical drawings, material inspection, visual inspection (clean status of components, sealing, foreign matter, firmness, assembly, etc.), dimensional inspection,

packaging, labeling, etc.

Each batch of semi-finished products and finished products has corresponding batch production records, which can trace the production history and quality.

2.3 Material control and supplier management

According to the characteristics of the single-use system, the selection criteria, management methods and audit specifications for suppliers have been established. The quality of raw materials and components used is strictly controlled. Raw materials and components for production are controlled according to risk levels. Typical raw materials and components should meet the following basic requirements:

- Meets USP<87> and USP<88> Class VI, and / or ISO10993
- TSE/BSE
- USP < 665 > Compliance
- Other Regulatory Compliance Documents

Before production, all raw materials and components need to pass the internal QC test.

Suppliers are regularly audited to review the quality of raw materials and components to ensure consistency and reliability.

2.4 Personnel

LePure Biotech has a group of production, quality, management and technical personnel with corresponding professional knowledge of single-use systems. Ongoing staff training and qualifications to ensure employees have awareness of the required standards. Employee qualifications are measured through rigorous initial screening and ongoing competency testing.

2.5 Change Management

Based on science and risk, LePure Biotech has fully evaluated the factors that affect the performance of the production process and the quality of the final product, and established a systematic change management strategy.

If there may affect product consistency and quality reliability, such as changes in film material

formulation or important production process parameters, in order to ensure that users have sufficient time for assessment, LePure Biotech will normally notify users at least 6 months in advance.

2.6 Gamma sterilization process

According to the requirements of ISO11137, VDmax25 method was used for dose setting and distribution experiment, and the daily gamma radiation dose range was finally determined to be 25~40kGy, so as to ensure the sterility guarantee level SAL < 10^{-6}. At the same time, dose verification experiment is carried out regularly to ensure continuous effectiveness of sterilization parameters.

2.7 Batch management and traceability management

The batch production protocols for LeCouple® KY Sampling Kits are established to ensure the homogeneity and consistency of the quality and characteristics of the same batch of products, and standard operation procedures are in place for the management of batch number and production date so that each batch of product has a unique traceable batch number.

The raw and auxiliary materials, final bulk and finished products, major equipment and necessary production workshops used for the LeCouple® KY Sampling Kits and mixing systems are labeled, or otherwise marked with names, specifications and batch numbers of materials in production, so as to trace the whole production process.

3. Test and evaluation

The LeCouple® KY Sampling Kit has completed complex biocompatibility, chemical, physical, freeze-thaw effect test, bacterial challenge test, extractable test and other tests, providing users with reliable performance in the biopharmaceutical process.

Depending on the nature of the validation method, validation is performed on individual components, representative test bags, or the final product containing all relevant components.

3.1 Integrity Test

Testing purposes

The purpose of this test is to check whether there is leakage in the LeCouple® KY Sampling kit. The key points of verification include: welding tightness between the mouth of the boat port and the bag, welding tightness of the bag itself, and integrity of the connection site.

Testing method

Table 1. Summary of test methods

Test content	Testing method
Integrity tests	The sampling kit is fixed with special tooling and connected to the integrity tester pipeline through the Luer joint. Open the integrity tester, press to a certain pressure, continue for a certain time, monitor the drop of pressure, and judge whether there is leakage of the bag body by the value of the pressure drop.
Test the air tightness underwater	The Sampling kit is connected to the compressed air source through the Luer joint. The Sampling kit is filled with air, and then immersed in water. The pressure of the bag body is maintained at no less than 0.6bar through the regulating valve for 1min.

Test result

1. Bag body integrity test:

Table 2. Statistical table of bag integrity test results

Cat. No	Quantity	Gamma Irradiation	Results
HS0250-3K	15	50-55KGy	Pass
HS0250-2K	25		Pass
HS0100-3K	25		Pass
HS0100-2K	15		Pass
HS0050-3K	30		Pass
HS0050-2K	20		Pass
HS001-3K	16		Pass
HS001-2K	44		Pass

2. Test the air tightness underwater

Table 3. Statistical table of integrity test results

Cat. No	Quantity	Gamma Irradiation	Results
HS0050-3K	4	50-55KGy	Pass
HS0050-2K	4		Pass
HS0100-3K	4		Pass
HS0100-2K	4		Pass
HS0250-3K	4		Pass
HS0250-2K	4		Pass
HS001-3K	4		Pass
HS001-2K	4		Pass

3.2 Blasting test

Testing purposes

The purpose of this test is to verify the welding between the boat port and the bag as well as the mechanical strength of the bag body itself.

Testing method

The blasting test was carried out according to the standard operating procedures of LePure Biotech. All the test samples were irradiated by 50-55KGy, and the test samples were deemed to have passed without blasting within 0.6bar.

Test result

Table 4. Statistical table of blasting test results

Cat. No	Quantity (PCS)	Gamma Irradiation	Results
HS0050-3K	4	50-55KGy	Pass
HS0050-2K	4		Pass
HS0100-3K	4		Pass
HS0100-2K	4		Pass
HS0250-3K	4		Pass
HS0250-2K	4		Pass
HS001-3K	4		Pass
HS001-2K	4		Pass

3.3 Pipe connection pulling force Test

Testing purposes

The purpose of this test is to verify the pulling force of the boat port and the hose connection, the sampling needle (1mm and 2mm) and the hose connection, and the sampling needle and the sealing cap after welding.

Testing method

Table 5. Summary of test methods

Test content	Testing method
Pulling force test of boat port and hose connection	Cut the needle sampling bag, only retain the ship-shaped bag port and the hose connection part, and then carry out the tensile test, the pull force is not less than 40N is considered to be passed.
Pulling force test of boat bag mouth and hose connection	Cut the needle sampling bag, only retain the ship-shaped bag port and the hose connection part, and then carry out the tensile test, the pull force is not less than 40N is considered to be passed.
Pulling force test result between sampling needle and sealing cap after welding	Take 16 sampling needles (1mm and 2mm) after welding for tensile test, the pull-off force is not less than 25N to pass.

Test result

1. Pulling force test of boat port and hose connection

Table 6. Pulling force test of boat port and hose connection

Sample quantity	Gamma Irradiation	Results
16	50-55KGy	Pass

2. Test result of pulling force between sampling needle and hose connection:

Table 7. Test results of pulling force between sampling needle and hose connection

Sample quantity	Gamma Irradiation	Results
16	50-55KGy	Pass

3. Test result of pulling force between sampling needle and sealing cap after welding:

Table 8. Tensile test results of sampling needle and sealing cap after welding

sampling needle ID	Sample quantity	Gamma Irradiation	Results
1mm	16	50-55KGy	Pass
2mm	16		Pass

Conclusion: All test items and pull force test results are within the standard requirements, and meet the design requirements.

3.4 High and low temperature storage test

Testing purposes

Verify the integrity (boat port and bag, and all connection parts) of LeCouple® KY Sampling Kit at a specific limit ambient temperature.

Testing method

Table 9. Summary of test methods

Test content	Testing method
ultra-low temperature storage test (-80°C)	Samples of all specifications were filled with liquid, which was repeatedly freeze-thawed twice at -80 °C . After returning to normal temperature, the liquid in the sample was emptied for underwater air tightness test. Withstand 0.6bar underwater, lasting for 1min, no bubble generation is passed.
High temperature storage test (60°C)	The sample was filled with liquid and placed at 60°C for 96 hours before taking out. After normal temperature was restored, the liquid in the sample was emptied for underwater air tightness test. Withstand 0.6bar underwater, lasting for 1min, no bubble generation is passed.

Test result

1. Test results of storage at -80°C:

Table 10. Summary of test results for storage at -80°C

Item number of the sample	Sample quantity	Gamma Irradiation	Results
HS0050-2K	5	50-55KGy	Pass
HS0100-2K	5		Pass
HS0250-2K	5		Pass
HS001-2K	5		Pass
HS0050-3K	5		Pass
HS0100-3K	5		Pass
HS0250-3K	5		Pass
HS001-3K	5		Pass

2. Test results of high temperature storage at 60°C:

Table 11. Test results of high temperature storage at 60°C

Item number of the sample	Sample quantity	Gamma Irradiation	Results
HS0050-2K	3	50-55KGy	Pass
HS001-2K	3		Pass

HS0050-3K	3		Pass
HS001-3K	3		Pass

Conclusion: In the storage range of -80°C to 60°C, temperature has no significant effect on the integrity of the sampling kit, which meets the design requirements.

3.5 Suspension Test

Testing purposes

The purpose of this test is to verify that the aseptic sampling system will not leak the under the actual use condition.

Testing method

The sampling kit was filled with a standard capacity of water, and then fixed on the holder, suspended for 24h. Observe sampling kit and holder. Disconnect the ring with a disconnecting clamp, drain the liquid in the sampling kit, and test the underwater air tightness; At the same time, there is no obvious damage to the holder, bag port, bag body, pipeline and each connection. sampling kit can withstand 0.6bar under water for 1min, and the passage is done if there is no bubble.

Test result

Table 12. Summary of suspension test results

Item number of the sample	Sample quantity	Gamma Irradiation	Results	
			Appearence	Underwater tightness test
HS001-2K	5	50-55KGy	1. The hose at the suspension position is obviously elongated 2. No other obvious damage	Pass
HS001-3K	5		1. The hose at the suspension position is obviously elongated 2. The plastic shell at the mouth of one sample needle is slightly deformed 2. No other obvious damage	Pass

Results analyses

1. Hose stretching: It is caused by pulling the bag body under full load for a long time, but the deformation does not affect the sealing test results, which is a non-critical risk point.
2. Deformation of plastic shell at the port of the sampling needle: it is caused by the force of the bag body under full load for a long time, but does not have any impact on the flow path of the sampling needle, which is a non-critical risk point.

In normal use, the sampling bag is usually hung empty for a long time, and the probability of hanging full samples for a long time is small, and the impact on the product will not be greater than that of this test.

Conclusion: In normal use, this product will not leakage due to long suspension, meet the design requirements.

3.6 Post-puncture tightness test

Testing purposes

The purpose of this test was to verify that the simulated static pressure inside and outside the tank poses

a risk of leakage to the sealing cap after the completion of the LeCouple® KY Sampling Kit.

Testing method

Table 13. Summary of test method

Test content	Testing method
Post-puncture tightness test (Simulate static pressure inside tank)	The sampling kit is cut and only the connecting part of the sampling needle and hose is reserved. According to the standard operation process, the reserved part (5 samples) is assembled with the holder, and the puncture process is completed. Then, the holder is connected to the end of the blasting equipment, keep to a certain pressure and maintained for a certain time.
Post-puncture tightness test (Simulation tank external static pressure)	The sampling kit is cut and only the connecting part of the sampling needle and hose is reserved. According to the standard operation process, the reserved part (5 samples) is assembled with the holder, and the puncture process is completed. Then the holder is connected to the end of the blasting equipment, keep to a certain pressure and maintained for a certain time.

Test result

1. Simulated static pressure inside the tank body, sealing test results after puncture:

Table 14. Summary of static pressure test results in simulated tank

Item number of the sample	Sample quantity	Gamma Irradiation	Results
HS001-2K	5	50-55KGy	No liquid leakage
HS001-3K	5		No liquid leakage

2. Simulated external static pressure of the tank, sealing test results after puncture:

Table 15. Summary of external static pressure test results of simulated tank

Item number of the sample	Sample quantity	Gamma Irradiation	Results
HS001-2K	5	50-55KGy	Bubble-free generation
HS001-3K	5		Bubble-free generation

Conclusion: Under normal use, the aseptic sampling system will not leakage, and it meets the design requirements.

3.7 Protein adsorption test

Testing purposes

At room temperature (22±4°C), the concentration variation trend of BSA stored in the sample bag for 30 days was measured to study the effect of sample bag material on protein adsorption.

Testing method

The samples were placed at room temperature (22±4°C) and sampled at time points greater than or equal to 1, 7, 14, 21 and 30 days, respectively, to detect the protein concentration in the samples. Concentration was detected at all sampling points,The deviation from the initial sample concentration within 89%-111% is considered to be passed.

Test result

The results of sampling at day 1, 7, 14, 21 and 34 were all within the range of concentration deviation, so the influence of sampling bag on protein adsorption was within the acceptable range.

3.8 Bacteria Challenge Test

Testing purposes

The objective of this test was to verify ability of LeCouple® KY sterile sampling system and to ensure the sterility of both the sampled system and the samples taken during multiple puncture sampling.

Testing method

All samples were sterilized by 50-55KGy gamma irradiation prior to testing. A bacterial challenge test of LeCouple® KY sterile sampling system was performed using 10^7CFU B. diminuta, ATCC 19146. In the environment of 10^7CFU biological load, the sampling kit was repeatedly pierced through the front silicone protective sleeve 50 times to test whether the inside of the sampling system could ensure sterility.

Test result

The LeCouple® KY Sampling Kit ensures internal sterility through 50 puncture challenges.

3.9 Pharmacopoeia compliance

3.9.1 USP<85> bacterial endotoxin test

The test standard for bacterial endotoxin in the LeCouple® KY Sampling Kit was <2.15EU/ unit.

According to CHP<1143> and USP<85>, The LeCouple® KY Sampling Kit passed all tests, in accordance with the Chinese Pharmacopoeia and the United States Pharmacopoeia as well as the Biointernal control standards for bacterial endotoxin control standards.

3.9.2 USP<788> insoluble particle test

The objective of this test was to investigate the level of insoluble particles in the LeCouple® KY Sampling Kit manufactured with LeKrius® membranes. In order to avoid excessive liquid loading, dilution of particles, resulting in deviation of test results, the enterprise standard has detailed provisions on the liquid loading amount.

The internal control standards of LePure Biotech for insoluble particles are as follows:

⩾10μm : ⩽ 10 psc / mL

⩾ 25μm : ⩽ 1 psc/ mL

According to the test requirements of CHP<0903> and USP<788>, LeThenea single use cryopreservation bag has passed all the tests, and can meet the control standards of CHP< 0903> and USP< 788>, as well as the internal control standards of LePure Biotech for insoluble particles.

3.10 Verification of sterilization by gamma irradiation

Biological single-use system includes three steps: product definition, process definition and dose field test. The implementation process is as follows:

Product definition

Select typical single-use system components for microbial load testing to obtain initial microbial load data.

According to the definition of ISO11137 regulations, the single-use system series products are classified as a product family during the irradiation sterilization process.

Process definition

Includes precise dosing of radiation, sterility testing methods, validation of bioburden recovery, and more. It proved that the pharmaceutical single-use system is suitable for the VD max 25 radiation sterilization dose establishment method, and the minimum sterilization dose of the product is 25kGy.

According to the radiation verification results of pharmaceutical single-use system products, during the Gamma sterilization process, there may be up to 1.6 times the unevenness of radiation, so the set radiation dose range is 25~40kGy.

Dose Field Distribution Test

LePure Biotech entrusts external irradiation processing enterprises to carry out gamma irradiation sterilization processing on products. The radiation dose field distribution test was carried out on the external irradiation device, and the test results showed that the radiation dose of the product in each irradiation device was in the range of 25~40kGy.

Conclusion

According to the above verification process, the results show that the irradiation sterilization dose of the

single-use system is set at 25~40kGy, which can reach the sterility assurance level of S AL < 10 -6.

3.11 Others

3.11.1 Biocompatibility

The LeCouple® KY Sampling Kit meet the biocompatibility requirements of USP<87> and USP<88>VI plastics.

3.11.2 Self-life

The LeCouple® KY Sampling Kit is valid for 2 years. A longer validity period test and an aging test simulating real conditions are in progress, and LePure Biotech will further update the data regularly according to the test results.

4. Conclusion

This paper summarizes the key quality attributes in the design, development, production, inspection and validation of LeCouple® KY Sampling Kits manufactured by LeKrius® membrane. For further needs, please contact LePure Biotech local sales representative for original data test report and other information.

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