SterilEnz®-II/AT

Standard Sanitary Fittings for Single-Use Systems

Validation Guide Summary

Rev.00 – 30 December 2016

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1.0 **Product Information**

1.1 Product Description

SterilEnz®-II/AT polypropylene connectors are the only sanitary flange fittings that come equipped with a medical grade silicone gasket pre-attached to the fitting face. This feature prevents operators from miss-aligning or dropping gaskets during assembly. The fittings can be sterilized via autoclave or gamma irradiation. SterilEnz-II/AT fittings work with any standard flange gasket as well. Simply remove the pre-attached gasket (no tools required) and install your own.

The SterilEnz®-II/AT connectors is produced from USP Class VI approved raw materials and is manufactured in an ISO 9001:2008 and ISO 13485:2003 registered facility under controlled environment in an ISO 14644-1 Class 7 clean room, in accordance with cGMP principles.

The SterilEnz®-II/AT connectors can be sterilized using gamma-irradiation (up to 40 kGy) and autoclave.

1.2 Product Testing

The SterilEnz®-II/AT tubing meets or exceeds the following regulatory requirements:

- USP 88 Class VI requirements; Post-autoclave and Post-gamma.
- USP 87 MEM Elution Cytotoxicity Test; Post-autoclave and Post-gamma
- USP 661 Physicochemical Test for Plastics; Post-autoclave and Post-gamma
- USP 788 Particulate Matter; Post-autoclave and Post-gamma

1.3 Shelf Life and Storage Recommendations

SterilEnz®-II/AT connectors should be stored in the original, unopened packaging in dry, moisture-free environment, at ambient temperature and away from direct outside weather influences. When stored in accordance with these recommendations, SterilEnz®-II/AT has a warranty of 5 years from date of manufacture.

1.4 Country of Origin

SterilEnz®-II/AT is manufactured in the U.S.A.

1.5 Animal Origin

The raw material used in the manufacture of SterilEnz®-II/AT is free of bovine or other animal derived content, based on the supplier information. In addition, animal or human-origin products or by-products, which may give rise to Bovine Spongiform Encephalopathy (BSE), and any other type of Transmissible Spongiform Encephalopathy (TSE) as defined in EMA/410/01 rev.3, are not used in the manufacturing process of SterilEnz®-II/AT.

2.0 Regulatory Compliance Information

2.1 Summary of Regulatory Compliance Information

Test	Standard	Result
Red Cell Hemolysis Test; Post-Gamma	ASTM	Passed
Red Cell Hemolysis Test; Post-Autoclave	ASTM	Passed
Biological Reactivity Tests, *In Vitro*: L929 MEM Elution, Post-Gamma	USP <87>	Passed
Biological Reactivity Tests, *In Vitro*: L929 MEM Elution, Post-Autoclave	USP <87>	Passed
Biological Reactivity Tests, *In Vivo*: Post-Gamma	USP <88>	Passed
Biological Reactivity Tests, *In Vivo*: Post-Autoclave	USP <88>	Passed
Physicochemical Tests for Plastics; Post-Gamma	USP <661>	Passed
Physicochemical Tests for Plastics; Post-Autoclave	USP <661>	Passed
Particulate Matter; Post-Gamma	USP <788>	Passed
Particulate Matter; Post-Autoclave	USP <788>	Passed
The post-Gamma tests were performed on SterilEnz®-II/AT samples subjected to 25-40 kGy gamma-irradiation.		
The post-autoclave tests were performed on SterilEnz®-II/AT samples subjected to Autoclave at 125 °C for 45 minutes.		

2.2 Red Cell Hemolysis; Post-Gamma and Post-Autoclave

Test Reference	American Society for Testing and Materials method F756-00 and G21 CFR 58 (GLP Regulations.)
Purpose	This study assesses the hemolytic activity of a SterilEnz®-II/AT in indirect contact with rabbit blood.
Procedure	The extraction was performed by immersing the test articles in endotoxin free water and placing it on an orbital shaker in an incubator for not less than 60 minutes at 37-40 °C. Standard controls and a positive product control demonstrated the compliancy of the assay.
Evaluation Criteria	The post-gamma and post-autoclave SterilEnz®-II/AT hemolytic activity was evaluated by indirect contact using ASTM method F756-00.
Results Post-Gamma	The post-gamma SterilEnz®-II/AT exhibited 0.18% hemolysis above the level of hemolysis exhibited by the negative control. The SterilEnz®-II/AT is considered non-hemolytic under the experimental conditions employed.
Results Post-Autoclave	The post-autoclave SterilEnz®-II/AT exhibited 0.18% hemolysis above the level of hemolysis exhibited by the negative control. The SterilEnz®-II/AT is considered non-hemolytic under the experimental conditions employed.

2.3 Biological Reactivity Tests *In Vitro*, L929 MEM Elution USP <87>; Post-Gamma and Post-Autoclave

Test Reference	USP <87> Biological Reactivity Tests, *In Vitro* and ANSI/AAMI/ISO 10993-5.
Purpose	Cytotoxicity *In Vitro* tests are a fast and sensitive method used to assess the biocompatibility of the test material when in contact with a specific cell culture. Given the demonstrated sensitivity to extractable cytotoxic test articles, mouse fibroblast L929 cells had been used for cytotoxicity studies historically.
Procedure	Duplicates of gamma irradiated and autoclaved SterilEnz®-II/AT samples, positive control (Natural Rubber) and negative control (Negative Control Plastic) samples were immersed in Serum-Supplemented (complete) Minimum Essential Medium (MEM) for 24 hours prior to testing and incubated at 37 °C for 48 hours, in a humidified atmosphere containing 5% carbon dioxide.
Evaluation Criteria	The cultures were evaluated for biological reactivity (cellular degeneration and malformation) on a scale of 0 to 4, with Grade 0 being no signs of reactivity and Grade 4 being severe biological reactivity. The observed cellular response for positive control (Grade 4) and negative control (Grade 0) confirmed the suitability of the test system.
Results Post-Gamma	Post-gamma SterilEnz®-II/AT showed no biological reactivity (Grade 0) in the L929 mammalian cells at 48 hours, post exposure to the test article extract. Based on the criteria of the protocol, gamma-irradiated SterilEnz®-II/AT is non-cytotoxic and meet the requirements of the USP guidelines.
Results Post-Autoclave	Post-autoclave SterilEnz®-II/AT showed no biological reactivity (Grade 0) in the L929 mammalian cells at 48 hours, post exposure to the test article extract. Based on the criteria of the protocol, autoclaved SterilEnz®-II/AT is non-cytotoxic and meet the requirements of the USP guidelines.

2.4 Biological Reactivity Tests *In Vivo*, USP <88>; Post-Gamma and Post-Autoclave

Test Reference	USP <88> Class VI; Acute Systemic Toxicity, Intracutaneous Irritation and Intramuscular Implantation Tests
Purpose	USP Class VI tests characterize the biological response of animals to plastics based on responses to a series of *In-vivo* tests.
Procedure	The standard test extracts (0.9% saline, 5% ethanol in saline, polyethylene glycol 400 and sesame oil) were made from gamma-irradiated and autoclaved SterilEnz®-II/AT at 70 °C for 24 hours. *Acute Systemic Toxicity Test* - The test extracts and the negative controls were injected to mice systemically. *Intracutaneous Irritation Test* - The test extracts and the negative controls were injected intracutaneously to rabbits. *Intramuscular Implantation Test* - Strips of gamma-irradiated and autoclaved SterilEnz®-II/AT and negative controls were implanted into paravertebral site of rabbits.
Evaluation Criteria	*Acute Systemic Toxicity Test* - The mice were observed for 72 hours for signs of toxicity. *Intracutaneous Irritation Test* - The rabbits were observed for 72 hours for evidence of tissue reaction. *Intramuscular Implantation Test* - The rabbits were observed for 1 week for signs of hemorrhage, necrosis, discoloration and infections.
Results Post-Gamma	The post-gamma SterilEnz®-II/AT met the requirements of the USP Class VI - 70 °C for biocompatibility.
Results Post-Autoclave	The post-autoclave SterilEnz®-II/AT met the requirements of the USP Class VI - 70 °C for biocompatibility.

2.5 Physicochemical Tests USP <661>; Post-Gamma and Post-Autoclave

<table>
<tr><td>Test Reference</td><td>USP <661> Containers, Physicochemical Tests-Plastics</td></tr>
<tr><td>Purpose</td><td>The physicochemical tests evaluate the physical and chemical properties of a plastic material.</td></tr>
<tr><td>Procedure</td><td>The post-gamma and post-autoclave SterilEnz®-II/AT samples were extracted in USP purified water for 70 °C for 24 hours.</td></tr>
<tr><td>Evaluation Criteria</td><td>The test extracts were tested for buffering capacity, non-volatile residue, residue on ignition and heavy metals.</td></tr>
<tr><td>Results</td><td>
<table>
<tr><th rowspan="2">Assay</th><th rowspan="2">Maximum Limit</th><th colspan="4">SterilEnz®-II/AT</th></tr>
<tr><th colspan="2">Post Gamma</th><th colspan="2">Post-Autoclave</th></tr>
<tr><th>Result</th><th>Pass/Fail</th><th>Result</th><th>Pass/Fail</th></tr>
<tr><td>Buffering Capacity</td><td>10.0 mL</td><td>0.6 mL</td><td>Pass</td><td>0.6 mL</td><td>Pass</td></tr>
<tr><td>Non-volatile residue</td><td>15 mg</td><td>0.0 mg</td><td>Pass</td><td>0.0 mg</td><td>Pass</td></tr>
<tr><td>Residue on ignition</td><td>5 mg</td><td>N/A</td><td>N/A</td><td>N/A</td><td>N/A</td></tr>
<tr><td>Heavy metals</td><td>1 ppm</td><td><1 ppm</td><td>Pass</td><td>< 1 ppm</td><td>Pass</td></tr>
</table>
</td></tr>
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2.6 Particulate Matter USP <788>; Post-Gamma and Post-Autoclave

<table>
<tr><td>Test Reference</td><td>USP <788> Particulate Matter in Injections</td></tr>
<tr><td>Purpose</td><td>Particulate Matter test provides information on the levels of extraneous, mobile, undissolved particles unintentionally present on the connector.</td></tr>
<tr><td>Procedure</td><td>The gamma-irradiated and autoclaved SterilEnz®-II/AT samples and control samples were flushed with Sterile Water for Injection (SWFI).</td></tr>
<tr><td>Evaluation Criteria</td><td>The rinsates of both gamma-irradiated and autoclaved SterilEnz®-II/AT were examined using light obscuration.</td></tr>
<tr><td>Results</td><td>
<table>
<tr><th rowspan="2">Particles/mL</th><th colspan="2">VWR®-SIL-C</th></tr>
<tr><th>Post-Gamma</th><th>Post-Autoclave</th></tr>
<tr><td>≥ 10 µm</td><td>7.65</td><td>7.65</td></tr>
<tr><td>≥ 25 µm</td><td>.65</td><td>.65</td></tr>
</table>
</td></tr>
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3.0 Legislations

3.1 Restriction of Hazardous Substances (RoHS)

In compliance with the RoHS in Electrical and Electronic Equipment Directive 2011/65/EU, SterilEnz®-II/AT does not use or intentionally add the following substances in the manufacture of SterilEnz®-II/AT tubing:

- Lead (Pb)
- Mercury (Hg)
- Hexavalent Chromium (CrVI)
- Polybrominated biphenyls (PBBs)
- Polybrominated diphenyl ethers (PDBEs)
- Cadmium (Cd)

3.2 REACH

Raw materials used in the formulation of SterilEnz®-II/AT comply with European Union directive, 1907/2006/EC (REACH).

4.0 Substances of Concern

4.1 Bisphenol A (BPA)

Formulations of raw materials and manufacturing process of SterilEnz®-II/AT do not use BPA.

4.2 Latex

Latex is not used in the manufacture and formulation of SterilEnz®-II/AT.

4.3 Phthalates

Phthalates or any phthalate based plasticizers are not used or intentionally added in the manufacturing process of VWR-SIL-C tubing and the raw materials of SterilEnz®-II/AT tubing is free from phthalates including, but not limited to the following: Bis(2-ethylhexyl) phthalate (DOP), Bis(2-ethlhexyl) phthalate (DEHP), Dibutyl phthalate (DBP), benzxyl butyl phthalate (BBP), Diisopentyl phthalate (DIDP), Diisononyl phthalate (DINP) and Di-n-octyl phthalate (DnOP).

4.4 Melamine

VWR does not use melamine in the manufacturing process and the formulation of raw materials of SterilEnz®-II/AT tubing.

4.5 Food Allergens

Materials used in the manufacture and formulation of SterilEnz®-II/AT do not contain major food allergens including soy, wheat or other gluten sources, lactose, eggs, fish, crustaceans, peanuts and tree nuts.

4.6 Other

In addition, the following substances are not utilized in the manufacturing processes or added intentionally to the formulation of raw materials of SterilEnz®-II/AT tubing:

- Polychlorinated terphenyls (PCTs)
- Polychlorinated biphenyls (PCBs)
- Non phthalate based plasticizers
- Colorants and pigments
- Residual solvents
- Brominated and non-brominated flame retardants
- Arsenic
- Asbestos
- Jatropha plant derivatives

5.0 Revision History

The current revision is Revision 00, released on December 30, 2016.