

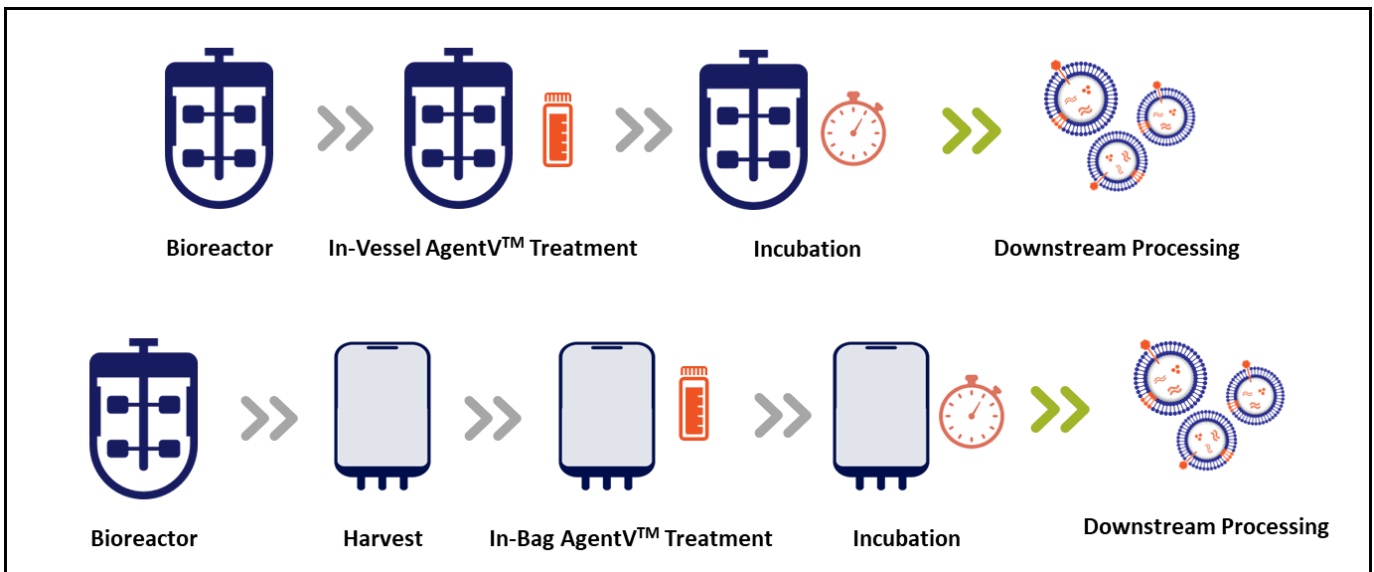
Recommended EV Conditioned Medium Treatment Protocol with AgentV™-DSP

Protocol Summary

AgentV-DSP (S46999), a treatment that contains recombinant biological molecules, solves DSP filtration problems by breaking up biological components in conditioned media and delaying filter fouling. When used at the harvest stage, it drastically improves the EV yield through normal flow filtration steps, increases the loading capacity per filter area, and facilitates greater turbidity reduction by enabling the use of lower, absolute retention rated filters with minimal impact to yield. AgentV-DSP can be used both in-vessel and in-bag prior to downstream process execution.

Process Overview

Protocol Summary



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Recommended In-Bag Treatment Protocol

1. AgentV-DSP Preparation

- 1.1. AgentV-DSP is shipped frozen, and, upon receipt, promptly store AgentV-DSP at -20°C.
- 1.2. Before use, thaw AgentV-DSP at room temperature for up to 4 hours, away from direct light.
 - 1.2.1. Alternatively, thaw AgentV-DSP at ~2-8°C away from for up to 36 hours.
- 1.3. After use, return AgentV-DSP to ~2-8°C away from light for up to 1 week from initial thaw.

2. Bioreactor Harvest

- 2.1. Harvest the treated conditioned medium into an appropriately sized, sterile bioprocessing bag following recommended harvest methods for the bioreactor used.
- 2.2. Obtain the volume of the harvested conditioned medium. This can be determined by assuming 1 g = 1 mL with a tared load cell.

3. AgentV-DSP Preparation

- 3.1. Calculate the amount of AgentV-DSP needed using the table below.

Harvest Volume (mL)		AgentV-DSP Dilution Factor		AgentV-DSP Volume Needed (mL)
	/	1000	=	

- 3.2. If the required AgentV-DSP volume is too low for reliable injection through addition lines, the AgentV-DSP can be diluted 10x with sterile EV collection medium to deliver the appropriate amount of AgentV-DSP using a larger addition volume.

4. In-Bag Treatment

- 4.1. Aseptically transfer the required AgentV-DSP volume to an appropriately sized bioprocess bag or assembly able to interface with the harvest bag.
- 4.2. Inject the AgentV-DSP immediately following harvest.
 - 4.2.1. Aseptically connect the AgentV-DSP vessel to the harvest bag and inject all the required volume.
- 4.3. Thoroughly mix the bag by gently massaging the sides.
- 4.4. Incubate for 30 minutes in a temperature-controlled incubator set to 37°C.
 - 4.4.1. Alternatively, incubation may occur at room temperature.
- 4.5. Continue with downstream processing as needed.

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Recommended In-Vessel Treatment Protocol

1. AgentV-DSP Preparation

- 1.1. AgentV-DSP is shipped frozen, and, upon receipt, promptly store AgentV-DSP at -20°C.
- 1.2. Before use, thaw AgentV-DSP at room temperature for up to 4 hours, away from direct light.
 - 1.2.1. Alternatively, thaw AgentV-DSP at ~2-8°C away from for up to 36 hours.
- 1.3. After use, return AgentV-DSP to ~2-8°C away from light for up to 1 week from initial thaw.

2. AgentV-DSP Preparation

- 2.1. Determine harvest volume of the bioreactor. This can be determined by assuming 1 g = 1 mL with a tared load cell or be estimated by bioreactor-specific working volume.
- 2.2. Calculate the amount of AgentV-DSP needed using the table below.

Harvest Volume (mL)		AgentV-DSP Dilution Factor		AgentV-DSP Volume Needed (mL)
	/	1000	=	

- 2.3. If the required AgentV-DSP volume is too low for reliable injection through media addition lines, the AgentV-DSP can be diluted 10x with sterile EV collection medium to deliver the appropriate amount of AgentV-DSP using a larger addition volume.

3. In-Vessel Treatment

- 3.1. Aseptically transfer the required AgentV-DSP volume to an appropriately sized bioprocess bag or assembly able to interface with the medium addition line of the bioreactor of choice.
 - 3.1.1. If the bioreactor is a bench-top system run inside a biosafety cabinet, it is acceptable to directly pipette AgentV-DSP inside the biosafety cabinet.
- 3.2. Inject the AgentV-DSP immediately prior to harvest.
 - 3.2.1. Aseptically connect the AgentV-DSP vessel to the bioreactor and inject all the required volume.
- 3.3. Thoroughly mix the bioreactor by returning agitation until a homogenous mixture is observed.
- 3.4. Turn off the agitation and incubate for 30 minutes.
 - 3.4.1. Leave temperature control on at 37°C during the incubation. If the temperature control requires a homogenous mixture to obtain accurate temperature measurements, turn off the temperature control to minimize risk of overheating the bioreactor.

4. Bioreactor Harvest

- 4.1. Harvest the treated conditioned medium following appropriate harvest methods for the bioreactor used.
- 4.2. Continue with downstream processing as needed.

Caution to Users: RoosterBio products contain human sourced materials and should be treated as potentially infectious. Employ universal safety precautions and wear protective clothing and eyewear while handling. Practice appropriate disposal techniques per CDC guidelines for biohazardous material.

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