Takara Bio Swedish Filial

Cellartis® MSC EV Wonder™ User Manual

Cat. No. Y51000
(160126)

Takara Bio Swedish Filial
A Takara Bio Company
Arvid Wallgrens backe 20, SE-413 46 Göteborg, Sweden
Europe Technical Support: techEU@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

Table of Contents

Table of Figures

Table of Tables

I. Introduction

Cellartis MSC EV Wonder is a complete chemically defined medium free from animal-derived components. It is specifically formulated to increase the production of extracellular vesicles (EVs) from mesenchymal stem cells (MSCs).

The procedures described in the manual relate to primary mesenchymal stem cell culture; protocol adjustments may be necessary depending on the cell line used. For further information on MSC culture, please refer to Cellartis MSC Xeno-Free Culture Medium User Manual

This product is intended for use by personnel trained in laboratory techniques and should be handled according to the principles of good cell culture practice. It's recommended to use the media and reagents specified in this manual to ensure optimal results. Please note that technical support may not be provided for cultures that do not follow the instructions outlined below.

II. List of Components

- Cellartis® MSC EV Wonder™ (Cat. No. Y51000)
  - 475 ml Cellartis® MSC EV Basal Medium (Cat. No. Y31002)
  - 25 ml Cellartis® MSC EV Supplement (Cat. No. Y31001)

III. Additional Materials Required

The following materials are required for the MSC culture and expansion, but not supplied:

- Cellartis® MSC Xeno-Free Culture Medium (Cat. No Y50200) or Cellartis® MSC Xeno-Free Culture Medium w/o Phenol Red (Cat. No Y50205)
- PBS Dulbecco's without Ca^{2+} & Mg^{2+} (D-PBS –/–)
- Accumax
- Sterile water
- Tissue culture-treated polystyrene cell culture vessels
- General cell culture equipment

The following materials are required, but not supplied, for the EV induction:

- PBS Dulbecco's without Ca^{2+} & Mg^{2+} (D-PBS –/–)
- General cell culture equipment
- Equipment or materials for EV purification or concentration

IV. General Considerations

A. Storage and Handling

Cellartis MSC EV Basal Medium (Cat. No. Y31002) should be stored at 2–8°C and used before the expiration date indicated on the label.

Cellartis MSC EV Supplement (Cat. No. Y31001) should be stored at −15°C to −25°C and used before the expiration date indicated on the label.

NOTE: The supplement should be thawed at room temperature (RT; 15–25°C) or overnight at 2–8°C. Do not thaw in a 37°C water bath.

NOTE: Once thawed, the supplement should be used immediately or aliquoted and stored at −20°C. After thawing an aliquot, use it immediately. Do not re-freeze. Do not use the supplement beyond its stated shelf life.

Complete Cellartis MSC EV Wonder may be stored at 2–8°C for up to 1 month.

V. Culture of MSCs in complete Cellartis MSC Xeno-Free Medium and EV production in Cellartis MSC EV Wonder

Figure 1 provides an overview of the workflow for thawing and expanding MSC lines in complete Cellartis MSC Xeno-Free Medium, followed by EV induction using Cellartis MSC EV Wonder.

We recommend performing EV induction from MSCs after the first passage and limiting the number of additional passages where possible.

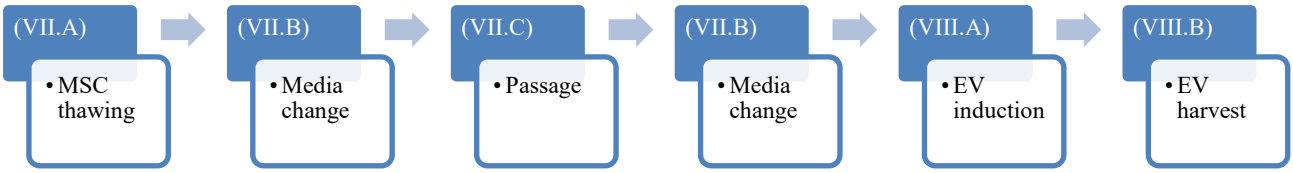


Figure 1. Schematic overview of the Cellartis MSC EV Wonder workflow. Corresponding sections of this user manual are indicated in brackets.

Cells should be cultured to a maximum confluency of 80–85%. A suggested end-to-end workflow, from MSC thawing to EV harvest, is depicted in Table I.

Table I. Suggested EV production schedule

Growth rate	Fast	Medium	Slow
Day 0	Thawing/seeding or subculture		
Day 1			
Day 2	Medium change	Medium change	
Day 3	Subculture		Medium change
Day 4		Subculture	
Day 5	Medium change		Subculture
Day 6	EV induction	Medium change	
Day 7			
Day 8		EV induction	Medium change
Day 9			
Day 10			EV induction
Day 11	EV harvest		
Day 12			
Day 13		EV harvest	
Day 14			
Day 15			EV harvest

NOTE: Always work under aseptic conditions.

VI. Preparing Cellartis MSC Culture Medium and Cellartis MSC EV Wonder

A. Preparation of Complete Cellartis MSC Culture Medium

1. Thaw Cellartis MSC Xeno-Free Supplement at 4°C or RT.
2. Decontaminate the external surfaces of reagents, including the medium bottle, using an appropriate disinfectant, and place them inside the biological safety cabinet.
3. Add the full volume (25 ml) of thawed Cellartis MSC Xeno-Free Supplement into Cellartis MSC Xeno-Free Basal Medium and mix thoroughly.
4. Store the complete medium at 4°C and use within one month. Do not refreeze.

NOTE: For more information, please refer to Cellartis MSC Xeno-Free Culture Medium User Manual

B. Preparation of Cellartis MSC EV Wonder

1. Thaw Cellartis MSC EV Supplement at 4°C or RT.
2. Decontaminate the external surfaces of all reagents and the medium bottle with an appropriate disinfectant, and place them in the biological safety cabinet.
3. Add 1 volume (e.g. 5 ml) of thawed Cellartis MSC EV Supplement to 19 volumes (e.g. 95 ml) of Cellartis MSC EV Basal Medium and mix thoroughly.
4. Aliquot any remaining Cellartis MSC EV Supplement and refreeze immediately.

VII. Thawing and Expansion of MSCs

When thawing MSCs for use in this workflow, seed at approximately 4.0–8.0 × 10^3 cells/cm^2 in 0.2–0.3 ml medium/cm^2.

NOTE: For more information, please refer to the Cellartis MSC Xeno-Free Culture Medium User Manual

A. Thawing Cells

NOTE—FOR YOUR PROTECTION: Wear a face shield and protective gloves. Use forceps when handling frozen vials. Never hold the vial directly in your hand, as rapid temperature changes may cause it to burst.

1. Pre-warm the complete Cellartis MSC Xeno-Free Culture Medium to between RT and 37°C.
2. Thaw frozen cells until a small piece of ice remains in the cryovial.
3. Transfer cells from the cryovial into a tube containing 5ml of complete Cellartis MSC Xeno-Free Medium. Rinse the vial with additional medium and add the rinse to the same tube.
4. Centrifuge at 200 × g for 5 minutes at RT.
5. Resuspend the cell pellet.
6. Seed cells at approximately 4.0–8.0 × 10^3 cells/cm^2 using 0.2–0.3 ml medium/cm^2 (see Table II).
7. Place the cell culture vessel in the incubator.

Table II. Recommended medium volumes and seeding densities for various cell culture vessels

Cell culture vessel	Cell-detachment reagent	Medium volume	Number of cells seeded at 4×10^3 to 8×10^3 cells/cm^2
12-well plate	0.4 ml/well	1 ml/well	1.5×10^4 to 3×10^4 cells
6-well plate	1 ml/well	2 ml/well	4×10^4 to 8×10^4 cells
T25 flask	2.5 ml	5 ml	1×10^5 to 2×10^5 cells
T75 flask	7.5 ml	15 ml	3×10^5 to 6×10^5 cells

B. Media Change

1. Check cells under a microscope and photograph as necessary for documentation.
2. Carefully aspirate the medium and add freshly warmed medium into the cell culture vessel. Avoid flushing medium directly onto the cell layer.
3. Return the cell culture vessel to the incubator.

C. Subculture and Expansion

1. Subculture when cells reach 70 to 80% confluency.
2. Prepare medium and cell culture vessels before harvesting the cells.
3. Aspirate the culture medium from the culture vessels and promptly wash with the same volume of DPBS−/− as the medium that was removed.
4. Aspirate the PBS. Add Accumax at 0.04 ml/cm^2.
5. Incubate for 5–7 at 37℃ and detach cells by gently tapping the side of the vessel.
6. Collect cells in a centrifuge tube. Rinse culture vessels using the same amount of Cellartis MSC Xeno-Free Culture Medium as cell detachment reagent added and collect it in the same centrifuge tube. Dilute the cell suspension with Cellartis MSC Xeno-Free Culture Medium (w/o Phenol Red), using 5 to 10 times the volume of cell detachment reagent added.
7. Centrifuge cells at 200 × g for 5 minutes at RT.
8. Resuspend the cell pellet.
9. Count cells.
10. Seed cells at approximately 4.0–8.0 × 10^3 cells/cm^2 with 0.2–0.3 ml medium/cm^2.
11. Return cell culture vessel to the incubator.

VIII. Induction of EV Production by MSC

We recommend initiating EV induction when the cells reach 65 to 85% confluency. For 65–75% confluency, use 0.2–0.3 ml of Cellartis MSC EV Wonder per cm^2 of culture surface. For >75% confluency, use 0.3–0.4 ml/cm^2.

A. EV Induction

1. Check cells under microscope; photograph as necessary for documentation.
2. Aspirate the medium from the cell culture vessel and wash the cell layer once with D-PBS (−/−).
3. Aspirate the D-PBS (−/−). Add 0.2–0.4 ml/cm^2 of Cellartis MSC EV Wonder to the cells according to the cell confluency.
4. Return the cell culture vessel to the incubator.

B. EV Harvest

1. After 5 days, check the cells under the microscope and document as necessary.
2. Collect the supernatant and proceed with further purification and characterization of EVs.

IX. Images of MSCs Maintained in Cellartis MSC Xeno-Free Culture Medium Before EV Induction

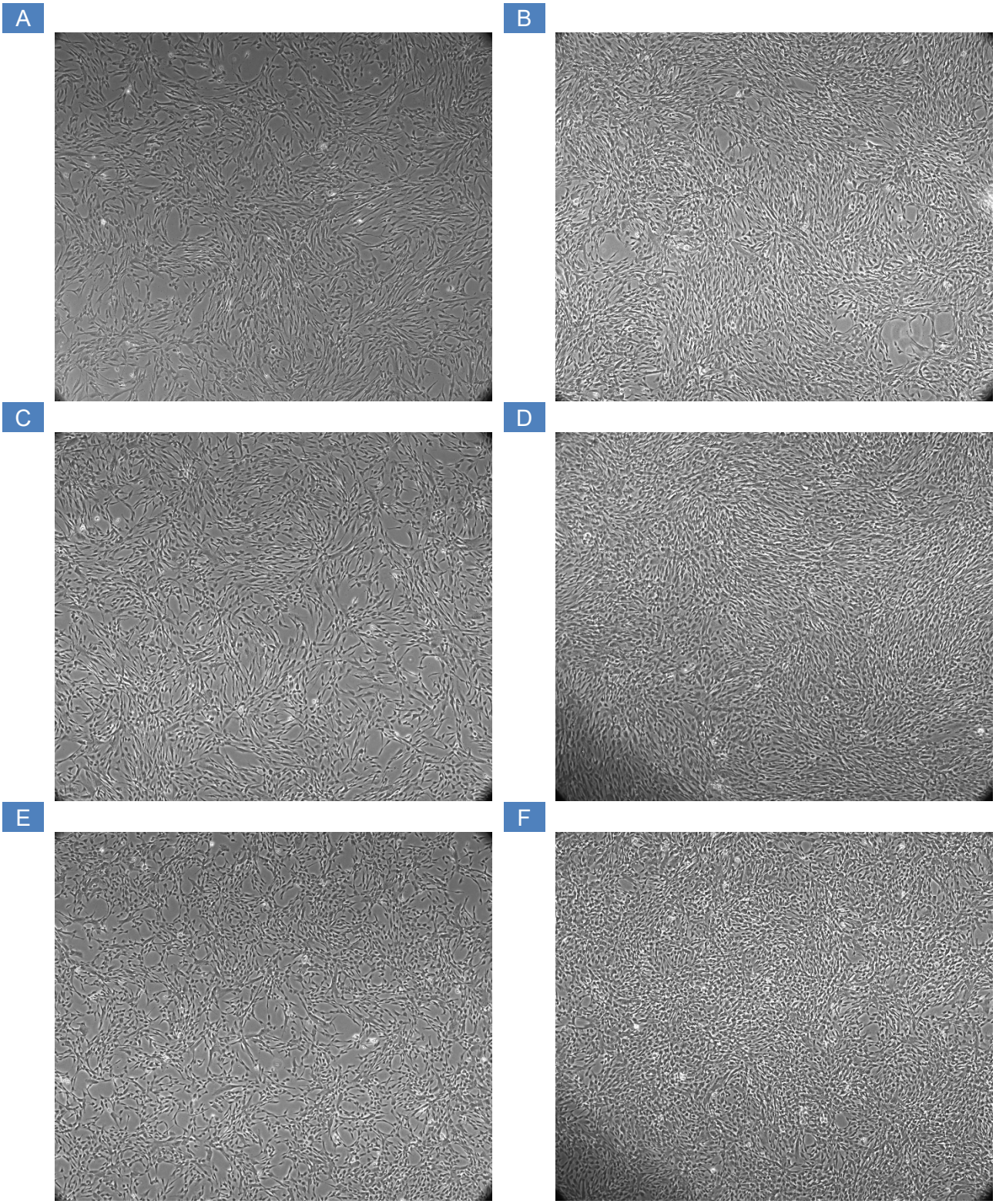


Figure 2. MSCs in complete Cellartis MSC Xeno-Free Culture Medium. Panels A–F: Representative images showing the recommended minimum (left) and maximum (right) confluency for passaging and EV induction across three MSC sources (4× magnification). **Panel A–B:** BM-MSCs. **Panel C–D:** AD-MSCs. **Panel E–F:** UC-MSCs.

X. Images of MSCs Maintained in Cellartis MSC Xeno-Free Culture Medium After EV Induction

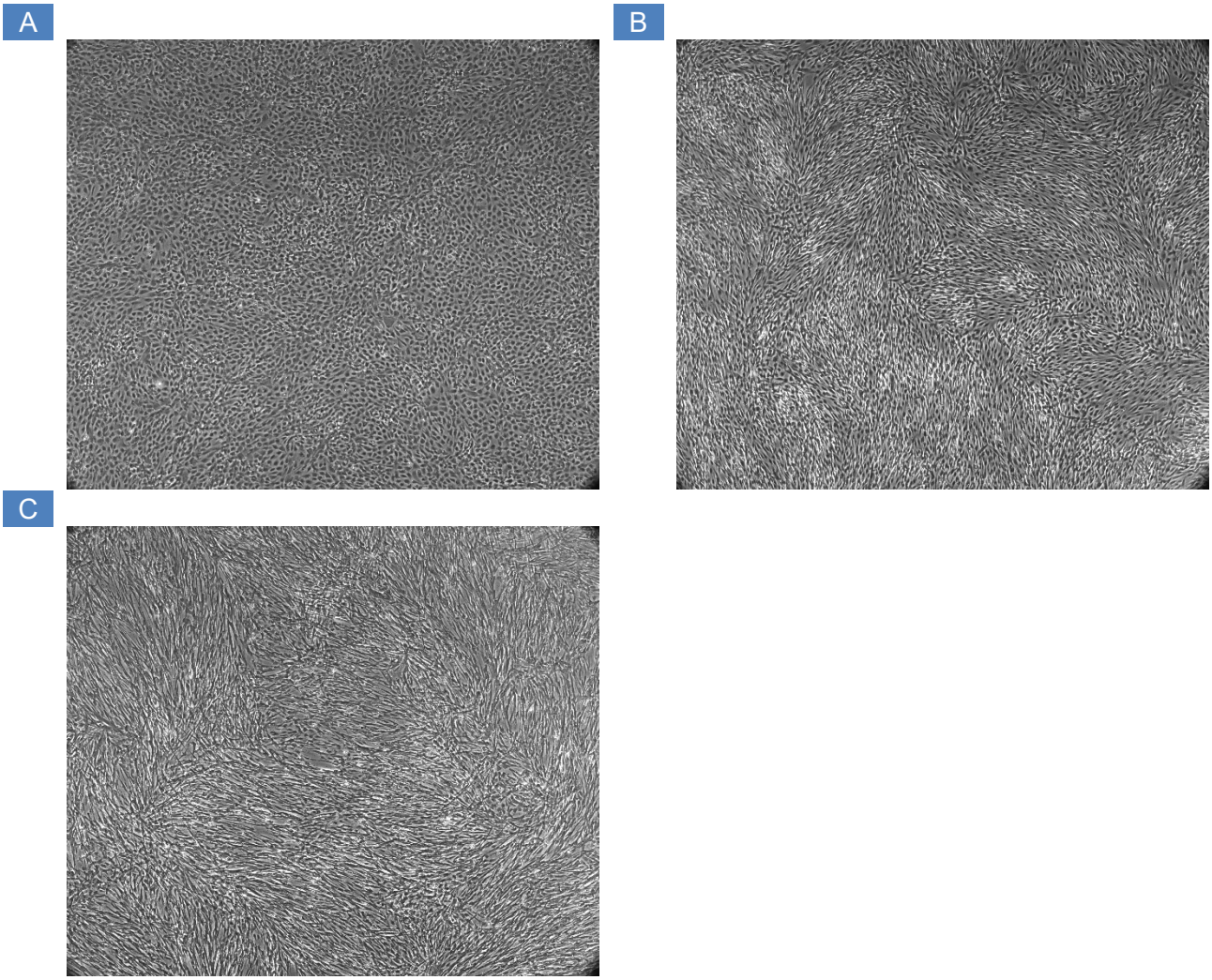


Figure 3. MSCs in complete Cellartis MSC EV Wonder after 5 days of EV induction. Panels A–C: Morphology of mesenchymal stem cells from different sources at 4× magnification. **Panel A:** BM-MSCs. **Panel B:** AD-MSCs. **Panel C:** UC-MSCs.

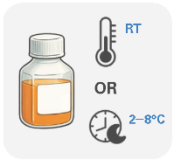
Appendix A. Troubleshooting Guide

Table III. Troubleshooting guide

Problem	Possible Explanation	Solution
Cells detach during culture.	MSCs require a coating reagent to adhere to the plastic.	Use Retronectin® coating as recommended in the Cellartis MSC Xeno-Free Culture Medium user manual.
Cells detach during EV induction.	The confluency of the MSCs was too high at the time of the induction.	Repeat the experiment with a lower seeding density or perform the EV induction 1–2 days earlier.
No EV production.	The cell concentration is too high relative to the volume of Cellartis MSC EV Wonder.	Increase the volume of Cellartis MSC EV Wonder used during the EV induction.

Appendix B. Protocol at a Glance

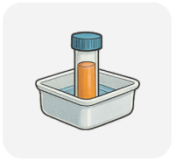
Preparation of Complete MSC Culture Medium



1 Thaw the MSC XF Supplement at room temperature (RT; 15–25°C) or overnight at 2–8°C

2 Add MSC XF Supplement to MSC XF Basal Medium at 1:20 dilution

Thawing of MSCs and Media Change



1 Thaw frozen cells

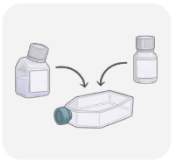
2 Transfer to a tube containing complete MSC XF Medium

3 Centrifuge at 200 x g for 5 mins at RT. Discard supernatant

4 Resuspend cells and plate into culture vessel

5 Change the media every 2 to 3 days using complete MSC XF Medium

Subculture and Expansion of MSCs



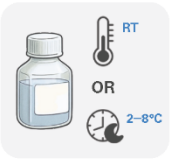
1 Aspirate medium. Rinse cells with D-PBS(–/–) and add Accumax

2 Incubate for 5 mins at 37°C. Collect detached cells in a tube with complete media

3 Centrifuge at 200 × g for 5 mins. Discard supernatant

4 Count and resuspend cells in complete media. Transfer to cell culture vessel

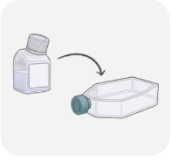
Preparation of Complete MSC EV Wonder



1 Thaw the MSC EV Supplement at RT or overnight at 2–8°C

2 Add MSC EV Supplement into the MSC EV Basal Medium at 1:20 dilution

EV Induction and Harvest



1 Rinse the cells with D-PBS(–/–)

2 Add Cellartis MSC EV Wonder

3 After 5 days, collect supernatant

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: +33.(0)1.3904.6880	tel: +33.(0)1.3904.6880
web: www.takarabio.com	web: www.takarabio.com
e-mail: ordersEU@takarabio.com	e-mail: techEU@takarabio.com

Notice to Purchaser

This product is to be used for Research Use or non-commercial manufacturing for clinical research. This product is not intended for human or animal in vivo applications. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

If you require licenses for other use, please contact us by phone at +33.(0)1.3904.6880.

Your use of this product is also subject to compliance with any applicable licensing requirements as detailed in our catalogues, on our website at http://www.takarabio.com, on the label or other documentation accompanying the goods. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.