INSTRUCTION MANUAL

EVA-AC-03-03-12

SUMILON™ Companion Plate for Cell Culture Inserts
for Use with EVOM™ Auto (24-Well)

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CONTENTS

ABOUT THIS MANUAL

The following symbols are used in this guide:

This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.

This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

INTRODUCTION

This document is a guide on how to set up the 24-well Auto Exchange Plate on the EVOM™ Auto Robot to be able to perform automated TEER measurements onto cell culture inserts.

Refer to EVOM™ Auto Instruction Manual to look for additional details of using EVOM™ Auto.

Refer to the EVOM™ Auto Install Guide Appendix A for detailed instructions on using the Expert mode in the EVOM™ Auto Software.

SETTING UP AUTO EXCHANGE PLATES WITH EVOM™ AUTO AND CELL CULTURE INSERTS

1. To be able to use the EVOM™ Auto, 24-well Auto Exchange Plate, and cell culture inserts, there are a few accessories that are needed. The 24M EVOM™ Auto Head, the C24/M24 Key will be needed to align the 24-well Auto Exchange Plate. Fig. 1 shows the accessories.

Fig. 1—Required accessories.

2. Install the 24M EVOM™ Electrode Array Head and C24/M24 EVOM™ Auto Plate Positioner. See Fig. 2 for their positions. Next, insert the 24-well Auto Exchange Plate into the plate holder slot. It is important to note that the indent of the plate should always be on the bottom right corner of the plate as shown in Fig. 3. The Auto Exchange Plate can only be used in one orientation. Use the indent on the corner of the plate as a guide to ensure the plate has been installed in the correct orientation. Again, if you are facing the EVOM™ Auto autosampler, the indent should remain on the front right corner when placed on the plate holder stage of the EVOM™ Auto. Make sure to pull back the spring-loaded lock on the right side as you insert the plate, and the Auto Exchange Plate should remain flat and firm on the plate holder (not unevenly elevated from the surface). See Fig. 4 for the complete assembly.

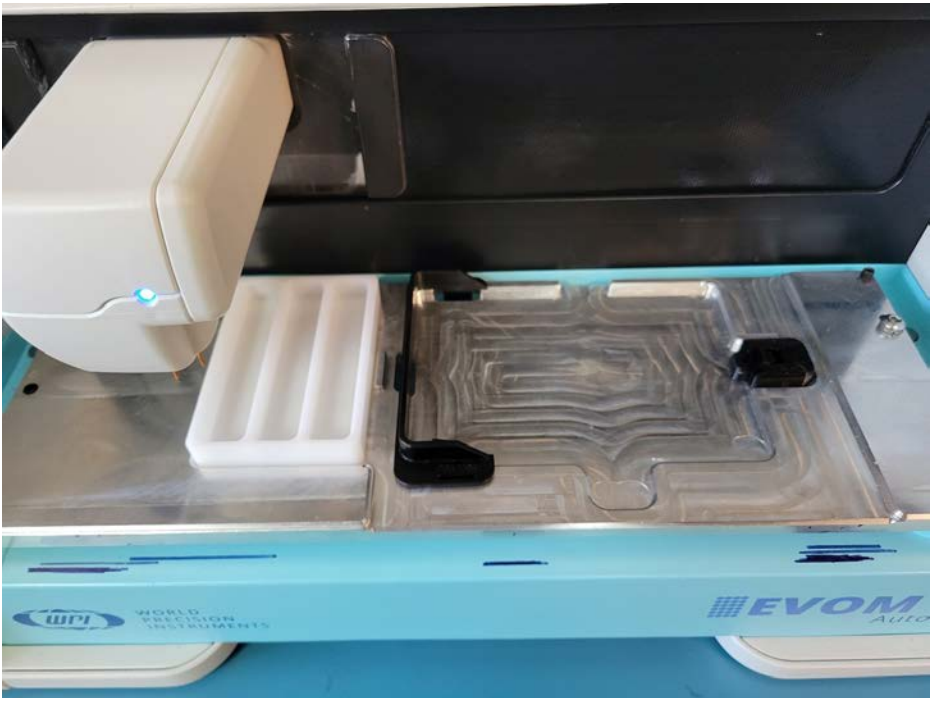

Fig. 2—24M EVOM™ Electrode Array Head and C24/M24 Plate Positioner inserted.

Fig. 3—24-well Auto Exchange Plate. Take note of the indent on the front right corner.

Fig. 4—Complete Assembly. The Indent should always be in this position to ensure correct plate orientation.

3. Looking at the Auto Exchange Plate wells, as seen in Fig. 5, there is a larger primary well area with numerous kinds of tabs that will act as the adapter for different kinds of inserts. This is where the front left or center or apical electrode of the EVOM™ Auto will be placed. The smaller well that's connected to the larger well is where the rear right or basolateral electrode will be placed. To make sure the electrodes are aligned with the 24-well Auto Exchange Plate, go to the Expert mode window in the EVOM™ Auto software. Then, click on travel position button in the well plate adjustments area. Use the left and right arrows to adjust the position of electrodes if or as needed. Refer to the Fig. 6 to see the alignment of the electrodes over the Auto Exchange Plate.

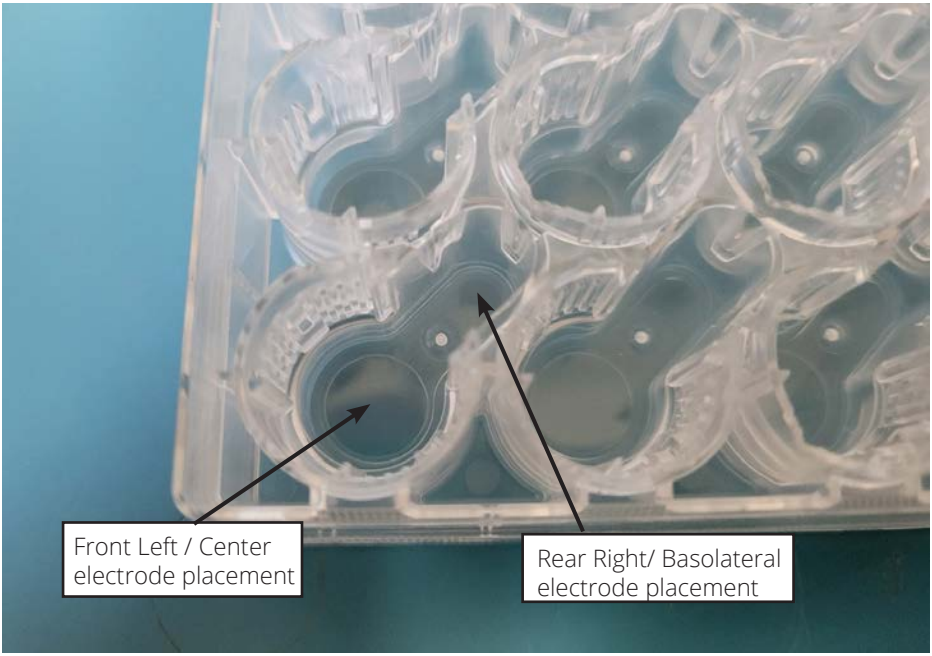

Fig. 5—Close-up of 24-well Auto Exchange Plate.

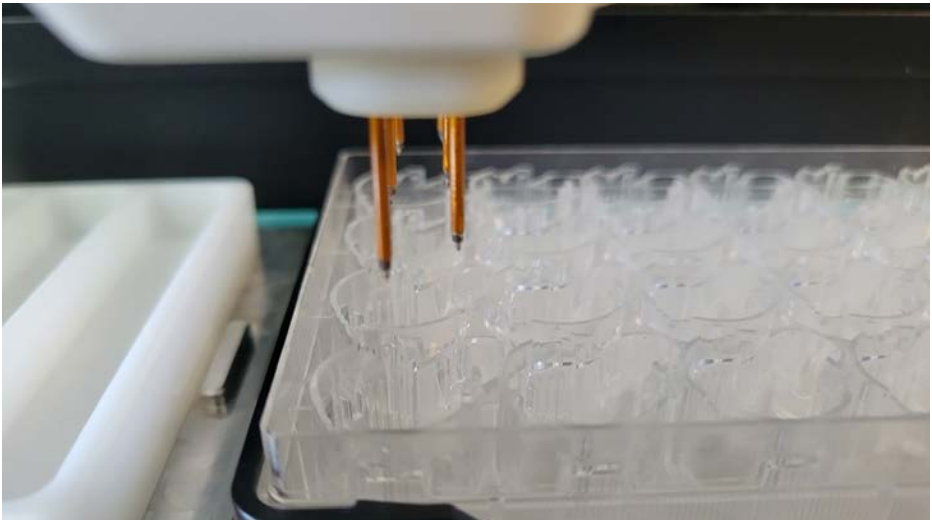

Fig. 6—Electrodes aligned over the Auto Exchange Plate.

The following steps need to be followed to use 24-well cell culture inserts on the Auto Exchange Plate. In this guide, the Corning 24 and MatTek 24 inserts have been used as examples with details on how to set them up. Please refer to Fig. 7 for Corning 24 and MatTek 24 cell culture inserts. Additional cell culture inserts from other manufacturers: Falcon, Millicell Hanging inserts, Greiner were also verified to be compatible with Auto Exchange Plate and EVOM™ Auto.

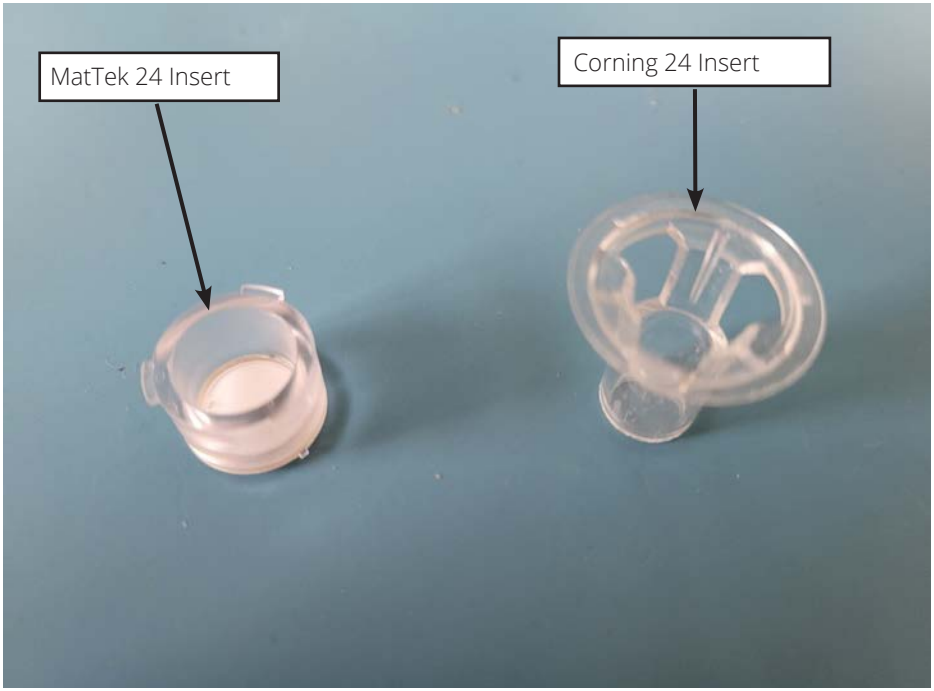

Fig. 7—MatTek and Corning 24-well Inserts

CAUTION: Before adjusting EVOM™ Auto in the expert mode with cell culture inserts, it is advised to work on a new profile or reset the current alignment settings to the factory default, then raise the electrode position to the measuring position to ensure that the electrodes don't penetrate through the membrane of the inserts. To do this, go to Expert window in the EVOM™ Auto software, click the measure position button in well plate adjustments area, and tap the Up button a few times.

USING WITH CORNING 24 INSERTS

1. For this section, the set up for Corning 24 inserts is shown. Carefully place a Corning insert into the larger well, as shown in Fig. 8. The orientation of the insert has no significance when using Corning 24-well inserts.

Fig. 8—Corning 24 inserts.

2. Click the Expert menu item on Main window of the EVOM™ Auto software. Click the travel position button in the well plate adjustments area and observe the position of the electrodes. The front left (center or apical) electrode should be positioned near the center of the Corning insert, and the rear right (basolateral) electrode should be in the lesser (basolateral) well of the Auto Exchange Plate, i.e., outside of the rim of the Corning insert. Adjust the positions with the left and right buttons if the electrode are not properly aligned. See Fig. 9, Fig. 10, and Fig. 11 for how the electrodes should be aligned for the Corning insert.

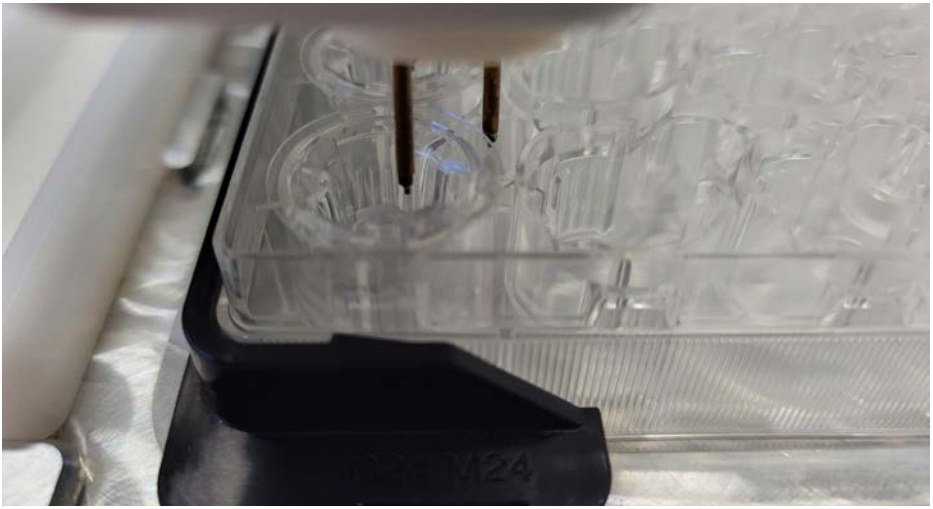

Fig. 9—Electrodes are aligned with the Auto Exchange Plate with Corning 24 inserts.

Fig. 10—Different angle of view of electrodes aligned with the Auto Exchange Plate with Corning 24 inserts.

3. While in the Expert window, click on measure position button in the well plate adjustments area. Now the electrodes should be inside the Corning insert and the Auto Exchange Plate. Use left and right adjustments as needed to properly align the electrode array, with the aim of having the front left (center) electrode close to the center of the insert, and with the back right (basolateral) electrode not making any contact with the rim of the insert. Adjust the up and down movement of the electrode by moving it close to the membrane of the insert but try not to enter too deep into the well, otherwise there is the possibility of damaging the membranes. See Fig. 11.

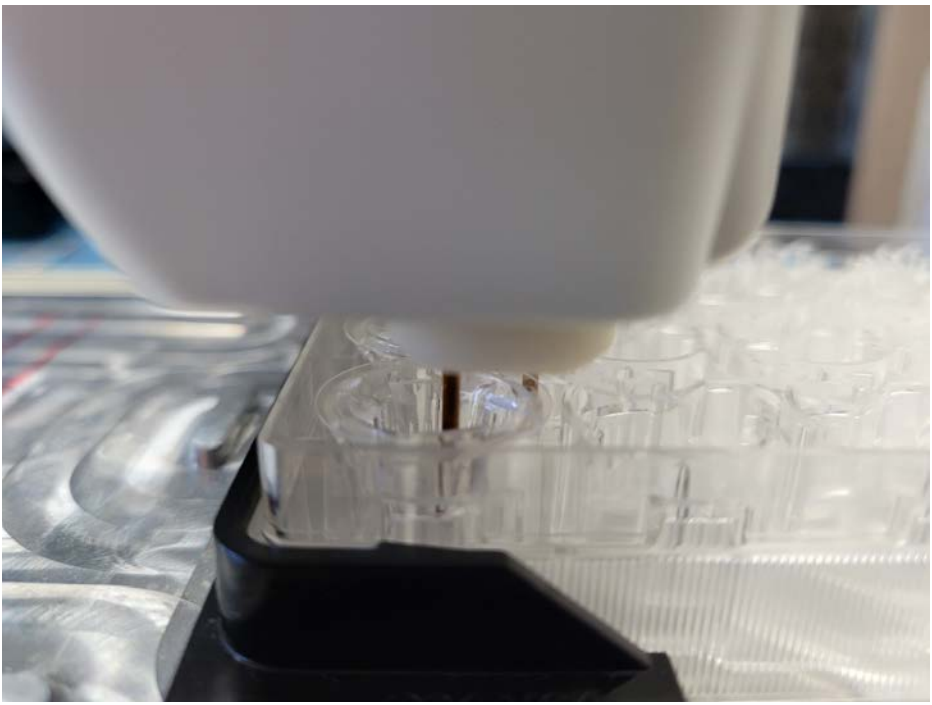

Fig. 11—Electrodes aligned with Corning 24 inserts. The electrodes have dropped down inside the inserts.

4. Make sure to save your settings and return to the Main window. Fill the insert and the auto exchange plate well with 160mM KCl solution. You may use sterile media or buffer to run this test instead of KCl solution. We recommend liquid volumes of 1200µL/1.2mL in the basolateral well of the Auto Exchange Plate and 200µL/0.2mL for the Corning insert. Select Experiment from the Main menu, and do a run for the specific Corning insert. If the measurement reading is shown as dashes (---), then the electrode wasn't deep enough to be in the solution. In this case, repeat step 3 and do a few more clicks down, then try again. If a number is read, it is advisable to do a few more runs to see if the reading varies. If the variation is large, then repeat step 3 and do a few more clicks down. If the numbers are consistent, then the EVOM™ Auto electrode array setting adjustment is complete for Corning 24 inserts. Now, you are ready to do experiments with 24-well Auto Exchange Plates and cell culture inserts.

USING WITH MATTEK 24 INSERTS

1. The MatTek 24 insert set up will follow a very similar principle as the Corning 24 insert. Take note of the three tabs on the upper rim of the MatTek insert. To successfully install the insert into the 24-well Auto Exchange Plate, have one of the three tabs pointed towards the back right of the well, where the opening that leads into the basolateral well. The tab should fit in the gap and the MatTek insert should slide down inside the well. See Fig. 12 for the proper positioning of the MatTek inserts. If the tab of the MatTek insert is not properly oriented, it will not slide down inside the Auto Exchange Plate, and the top surface will not be even with the surface of the Auto Exchange Plate. Fig. 13 shows the MatTek insert improperly positioned inside the Auto Exchange Plate.

Fig. 12—MatTek 24 insert in the Auto Exchange Plate.

Fig. 13—MatTek 24 insert improperly placed.

2. Select Expert from the Main menu to access the Expert window. Click on the travel position button in the well plate adjustments area and observe the position of the electrodes. The front left (center) electrode should be positioned near the center of the MatTek insert, and the rear right (basolateral) electrode should be in the basolateral well of the Auto Exchange Plate, outside of the edge of the MatTek insert. Adjust the positions with the left and right buttons as needed if the electrode is not properly aligned. See Fig. 14 for the electrodes being aligned to the MatTek insert.

Fig. 14—Electrode aligned with the MatTek 24 insert.

3. While you are on the Expert window, click on the measure position button in the well plate adjustments area. Now the electrodes should be inside the MatTek insert and the Auto Exchange Plate. Use left and right adjustments as needed, with the aim of having the front left electrode close to the center of the insert, and with the back right electrode not making any contact with the outside edge of the insert. Adjust the up and down movement of the electrode, by moving it close to the membrane of the insert, but try not to enter too deep into the sample insert. The membrane (in the insert) can be punctured by the electrode. Keep electrode 1 mm above the membrane. Please refer to Fig. 15.

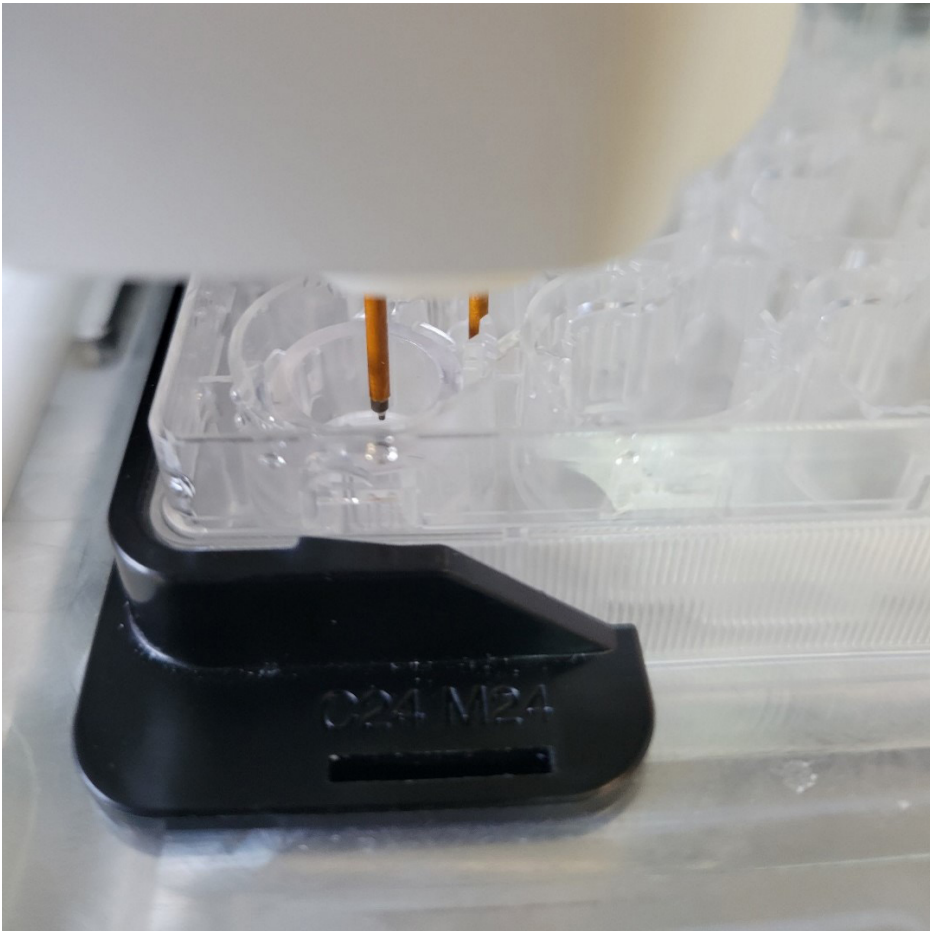

Fig. 15—Electrodes aligned with MatTek 24 inserts. The electrodes dropped down inside the inserts.

4. Save your settings and return to the Main window. Fill the insert and Auto Exchange Plate well with 160mM KCl solution. You may use sterile media or buffer to run this test instead of KCl solution. We recommend a basolateral liquid volume of 1750µL/1.75mL in the basolateral chamber of the Auto Exchange Plate, and 300µL/0.3 mL in the apical MatTek insert. Go to experiment mode and do a run for the specific MatTek insert. If the measurement reading is shown as dashes (---), then the electrode tips were not deep enough to be in the solution. In this case, repeat step 3 and do a few more clicks down, then try again. If a number is read, it is advisable to do a few more runs to see if the reading varies significantly. If the variation is large, then repeat step 3 and do a few more clicks down. If the numbers are consistent, then the EVOM™ Auto is ready to be used with MatTek 24 inserts and the Auto Exchange Plate. Now, you are ready to do experiments.

COMPATIBLE INSERTS & RECOMMENDED VOLUMES

Please follow the recommended liquid volumes mentioned in the table below to use 24-well cell culture inserts inside the Auto Exchange Plates and measure TEER with EVOM™ Auto.

24-well Insert Type	Example Part Number	Min. Apical Volumes Required for Auto Exchange Plates (mL)	Min. Basolateral Volumes Required for Auto Exchange Plates (mL)
Corning Costar	3470	0.30	1.25
MatTek	CCI24-PTFE-0.4-s	0.30	1.75
Millicell (hanging)	PTHT24H48	0.30	1.00
Falcon	353095	0.30	0.90
Greiner Bio-One	662641	0.30	1.00

CAUTION: DO NOT try to put different manufacturers' inserts in one single Auto Exchange Plate and attempt to measure with EVOM™ Auto. The settings of the EVOM™ Auto (electrode array positioning) with different insert types could be very different. Only use one single manufacturer's type of inserts in one single Auto Exchange Plate while using with EVOM™ Auto.