Appendix A: Preprogrammed Methods Files

There are some preprogrammed method files located in the **C:/Envision/common** folder. These are intended to be used as templates in making new method files. Each file controls the autosampler in different ways using the "user program" section of the AS control section of a method.

The autosampler can make injections using different modes. There are advantages and disadvantages to each. For detailed descriptions, please refer to the AS-700 Autosampler User Manual.

Application	**Recommended Method Template**
All applications not mentioned below. (default)	**AS700EasyMicroliterPickup_v1.MET**
Glu/GABA by FA-3ODS or other OPA derivatization methods	**AS700AminoAcidOPA_v4_14.MET**
Glu/GABA by FA-3ODS when ELS-500 switching valve is installed	**AS700AminoAcidOPA-ELS500_v4_14.MET**
Online Glutamate (GU-GEL column) with enzyme reactor and *using internal standard*	**AS700DoubleInjection90sDelay_v1.MET**
Online Glutamate (GU-GEL column) with enzyme reactor and *using external standard*	**AS700EasyMicroliterPickup_v1.MET**
ENO-30 users may benefit if enough sample	**AS700PartialLoop_v1.MET**
When you need to check that needle touches bottom of well or vial	**AS700CheckNeedleHeight_v1.MET**

AS700EasyMicroliterPickup_v1.MET

This is the main method that Eicom recommends. It will accept 5-50uL injections volumes and you can use water or methanol:water solutions as wash in the autosampler. If you need to inject smaller volumes, put the same mobile phase as you are using in the HPLC in the autosampler wash bottle and use the Microliter pick up method. If you need large volumes, install a large sample loop in the autosampler. Typically, you can only inject ½ the total volume of the sample loop.

AS700CheckNeedleHeight_v1.MET

This method is used to confirm the needle height setting. For maximum recovery of sample, the needles should just touch the bottom of the well or vial.

1. Put your plate or vials in the autosampler.
2. Make a sequence file.
3. Enter one of the front wells or vial positions in the sequence and select this method.
4. Run the sequence.
5. After the needle goes to the destination, pull back the spring loaded needle stripper mechanism and lift up the plate with your hand. You should be able to tell if the needle is touching the bottom or has some space between the needle and bottom of the well of vials.

Please note: The needle could also be too far down and poke through the bottom of the well or vial. To confirm proper setting, repeat procedure with higher needle height setting.

AS700DoubleInjection90sDelay_v1.MET

This method makes double injection 90s apart. Use for injecting an internal standard. We use for our online Glutamate method. Place samples in left plate and internal standard (glutamate in this case) in the corresponding wells of the plate on the right side. Same volume is injected during the second injection.

AS700AminoAcidOPA_v4_14.MET

This method is used for OPA derivitization of amino acids. It picks up a 4 uL of OPA solution from the right side plate and dispenses it to the sample in the corresponding well of the plate on the left . Then, it mixes 4x and waits 2.5 mins before injecting by the EMP method.

To use this method, place **exactly 16uL** of microdialysis sample or pH controlled homogenate sample in the left plate. If you have less than 16uL, please add solution to bring to 16uL. We do not have a specific recommendation for the diluant, but the carbonate buffer used to dilute OPA should work. On the right side plate, put **at least 20uL of 4mM OPA** solution in each well corresponding to the samples on the left side plate. In the sequence that uses this method, set injection volume to 10uL. Slightly more may be injected, but each sample well contains only 20uL of fluid at this point. You must leave safe margin to avoid injecting air or causing inconsistent results.

Autosampler temp is set to 10 degrees Celsius.

AS700AminoAcidOPA-ELS500_v4_14.MET

This is the same as previous method “AS700AminoAcidOPA_v4_14.MET” but with the addition of controls for switching valve timing.

AS700PartialLoop_v1.MET

This method theoretically has the highest accuracy. There is an extra flush step which flushes the needle with sample to remove the last traces of autosampler wash solution before drawing sample into loop. This volume is a minimum of 35 µL and is the amount of sample wasted during each injection. If you have a large volume of sample, this method may be the best method for you. If you are going to inject 10 ul of sample, you will need to have at least 50 µL in each well. 50 = 10 sample + 35 flush + 5 margin left in well for dead volume.

AS700lessthan5uLpickup_v1.MET

For accuracy with very small volume (less than 5 µL), you need to use this method. However, in order to use this method, you will need to place the same batch of mobile phase into the autosampler wash bottle. A segment of wash solution will be pulled into the sample loop on each side of the sample, and then injected along with the sample. This method will work well, but there are a couple of disadvantages. Seal life can be reduced because of the precipitate of salt from the mobile phase if the autosampler is not rinsed out after each use. Salt can also build up around the transport reservoir, requiring more frequent cleaning.