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CATALOG NUMBERS: K004-MAMAB-0001KT

Multi-Attribute Method (MAM) Mini Kit



The **96-well Multi-Attribute Method (MAM) Mini Plate Kit** standardizes the rapid analysis of biological medicines at every stage of manufacturing, eliminating the need for protocol development. The kit fully removes a broad range of contaminants, including detergents, salts, buffers, stabilizers, excipients, and others from up to 96 samples containing 10 µg – 250 µg of biological drug sample. The kit is designed to minimize or eliminate deamidation and chemical artifact formation.

Additional Reagents/Solutions (Not Included)

Liquid Chromatography-Mass Spectrometry (LC-MS) grade is recommended. where applicable

- Biological drug sample **(10 µg - 250 µg)**
- Protease of choice (e.g. Trypsin; 1 µg per 10 µg sample)*
- LC-MS grade Water
- LC-MS grade Methanol (MeOH)
- LC-MS grade Acetonitrile (ACN)
- LC-MS grade Formic Acid (FA)

* **Proteases are susceptible to freeze-thaw cycles.**

Equipment/Materials (Not Included)

- Single- and/or multi-channel pipettes (P2 through P1000) and corresponding tips
- Centrifuge capable of processing 96-well plates at 2000 x g
- Vortex mixer
- Heat block (adjusted to 55 °C)
- pH paper (pH < 1, optional)
- Humidified incubator (adjusted to 47 °C)
- Lyophilizer or SpeedVac
- Positive pressure apparatus or vacuum manifold (optional)
- Sonicator (optional, recommended)

Contents: (1) MAM 96-well Mini Plate, (1) 2.0 mL 96-well plate, (1) 1.0 mL 96-well plate, (1) silicone mat

①	Solution 1	2x Lysis Solution	2 x 1.5 mL	Strong detergent to solubilize sample
②	Solution 2	Reductant	1 x 200 µL	Reductant to break disulfide bonds
③	Solution 3	Alkylator	1 x 250 µL	Alkylator to prevent disulfides from reforming (mass shift is + 45.988, addition of SCH_2)
④	Solution 4	Acidifier	1 x 550 µL	Acidic solution to lower sample pH*
⑤	Solution 5	Binding/Wash Solution	4 x 3.0 mL (30.0 mL bottles)	Volatile buffer with alcohol to facilitate protein trapping and cleaning **Note: 27.0 mL LC-MS grade MeOH must be added to each bottle before use**
⑥	Solution 6	TruPROT™ Solution	2 x 1.5 mL	Engineered digestion buffer formulated to reduce deamidation and formation of adducts; contains 10% organic
⑦	Solution 7	Elution Solution	1 x 2.0 mL	Elutes peptides

All provided solutions, including Binding/Wash Solution with MeOH added, can be stored 1 month at 4 °C or up to 1 year at -20 °C. After thawing, mix thoroughly to ensure any precipitants are redissolved.

**** Acidifier must be stored tightly capped; it will degrade upon atmospheric exposure.***

RT = room temperature (20-25 °C), w/w = weight-to-weight ratio

Protocol:

① **1.** **Denature Protein.** To 23.0 µL of liquid sample, add 23.0 µL of **Solution 1 (2x Lysis Solution)**. For solid samples, dilute **Solution 1 (2x Lysis Solution)** to 1x by adding an equal volume of LC-MS grade water. Add 46.0 µL of 1x Solution 1 to a solid sample. Sonicate or vortex to fully solubilize and denature proteins. Samples can be processed in tubes or a deep well 96-well plate.

2. If sample is viscous due to the presence of DNA, sheer it thoroughly by probe sonication or enzymatically with a nuclease such as Benzonase™. Unsheared DNA will clog the protein trap.

3. Clarify sample as desired by centrifugation (e.g. 13,000 x g for 8 minutes). Transfer clarified lysate to tubes or a deep well 96-well plate. Pellet can be analyzed separately.

② **4.** **Reduce.** If desired, add 2.0 µL of **Solution 2 (Reductant)**. Incubate at RT for 10 minutes.

③ **5.** **Alkylate.** If desired, add 2.5 µL of **Solution 3 (Alkylator)**. Incubate at RT for 10 minutes.

⑤ **6.** While the sample is incubating, add 27.0 mL of LC-MS grade MeOH to a bottle of **Solution 5 (Binding/Wash Solution)**. Mix thoroughly.

④ **7.** **Acidify.** Add 5.0 µL of **Solution 4 (Acidifier)** to the sample. pH paper can be used to ensure pH ≤ 1. Proceed to the next step immediately.

⑤ **8.** Add 350 µL of **Solution 5 (Binding/Wash Solution) with MeOH added** to the sample. Mix thoroughly.

Note: *The sample may appear translucent at this step due to colloidal protein formation. Do NOT centrifuge.*

9. Place an **MAM 96-well Mini Plate** atop the 2.0 mL 96-well plate. Transfer each sample, including any insoluble material, into a well of the **MAM 96-well Mini Plate**.

Note: *No pre-equilibration is necessary. Solution may begin to drip through immediately; this is expected. The well can hold ~450 µL of solution. For larger volumes, the well can be loaded multiple times with the solution from Step 8. When transferring into the well, do not disturb the matrix.*

10. **Trap Protein.** Centrifuge the **MAM 96-well Mini Plate** and waste plate at 2,000 x g for 2 minutes. Visually confirm that all solution passed through the well. If not, centrifuge again for 5 minutes until no liquid remains. Discard flow-through and return the plate to the 2.0 mL 96-well plate.

Note: *A vacuum manifold or positive pressure can be used to draw solution through the plate provided that all wells have flow behavior, which might not be the case for all samples.*

⑤ **11.** **Clean Protein.** Add 200 µL of **Solution 5 (Binding/Wash Solution)** to the **MAM 96-well Mini Plate** and centrifuge at 2,000 x g for 2 minutes. Visually confirm that all solution passed through the well. Repeat washes 3 times; discard flow-through as necessary. Return the plate to the 2.0 mL 96-well plate.

Note: *Additional wash(es) may be performed if desired. Washes may be captured with the flow-through. If the sample has particularly hydrophobic contaminants, wash bound proteins 3 times with 450 µL 50% $CHCl_3$/50% MeOH, then perform 3 normal washes Solution 5 (Binding/Wash Solution).*

12. Move the **MAM 96-well Mini Plate** atop the 1.0 mL 96-well plate for collection.

⑥ **13.** Dilute trypsin in **Solution 6 (TruPROT™ Solution)** to a final volume of 30.0 µL, ensuring that the amount of protease in this solution is at a 1:10 (w/w) ratio with the total amount of protein in the sample (e.g. 10 µg per 100 µg of sample).

14. Transfer the entire 30.0 µL of trypsin solution to the **MAM 96-well Mini Plate**, ensuring the matrix is fully covered by digestion solution.

⑥ **15.** **Incubate & Digest.** Loosely cover the **MAM 96-well Mini Plate** to limit evaporative loss without making an air-tight seal. Place the plate atop the 1.0 mL 96-well plate in a 37 °C humidified incubator for 30 minutes.

Note: *Some dripping may occur during incubation; this is not of concern. Do NOT shake. If evaporation is significant during incubation, add 10.0 µL Solution 6 (TruPROT™ Solution).*

⑦ **16.** **Elute 1.** Add 20.0 µL of **Solution 7 (Elution Solution)** to all wells of the **MAM 96-well Mini Plate** atop the 1.0 mL 96-well plate after incubation. Centrifuge at 2,000 x g for 2 minutes or until all solution has passed through.

17. **Elute 2.** If hydrophobic peptides are of interest, add 20.0 µL of 50% (v/v) LC-MS grade ACN in 0.2% (v/v) LC-MS grade FA (solution not provided) to all wells of the **MAM 96-well Mini Plate**. Centrifuge at 2,000 x g for 2 minutes.

18. If Step 17 is performed, final sample will contain ~25% ACN. SpeedVac and resuspend in Aqueous Buffer A such as 5% ACN, 0.1% formic acid or dilute to < 5% ACN.

19. Sample ready for MS analysis.