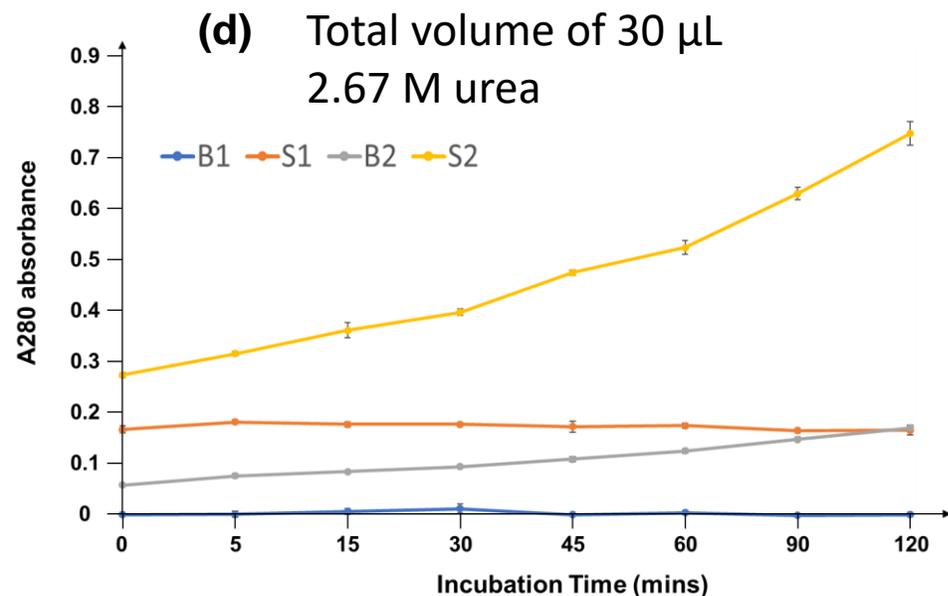
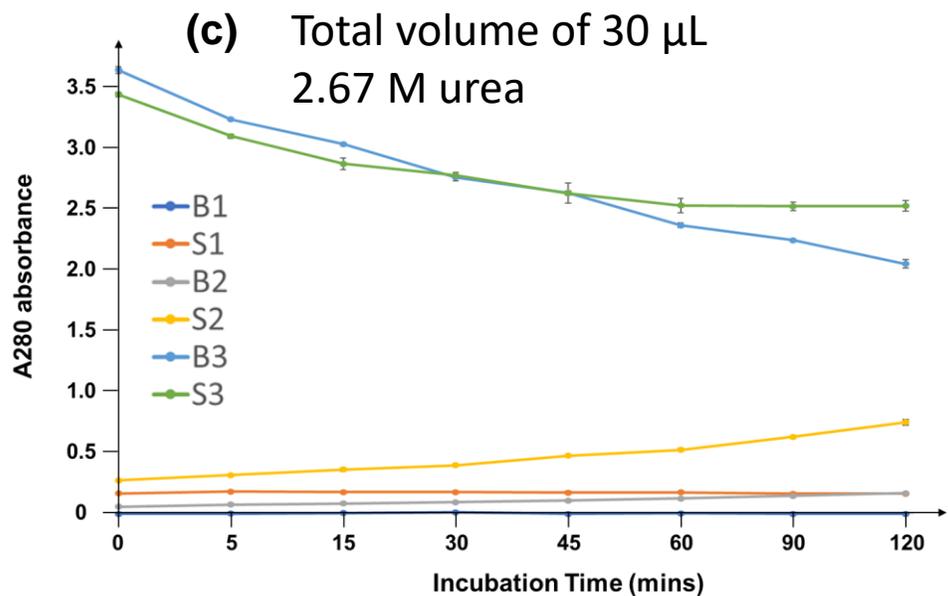
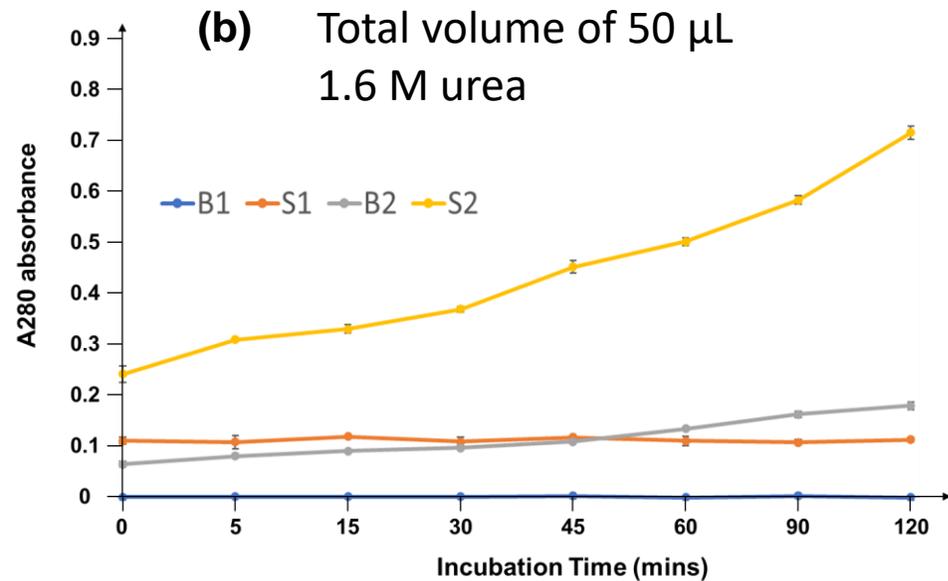
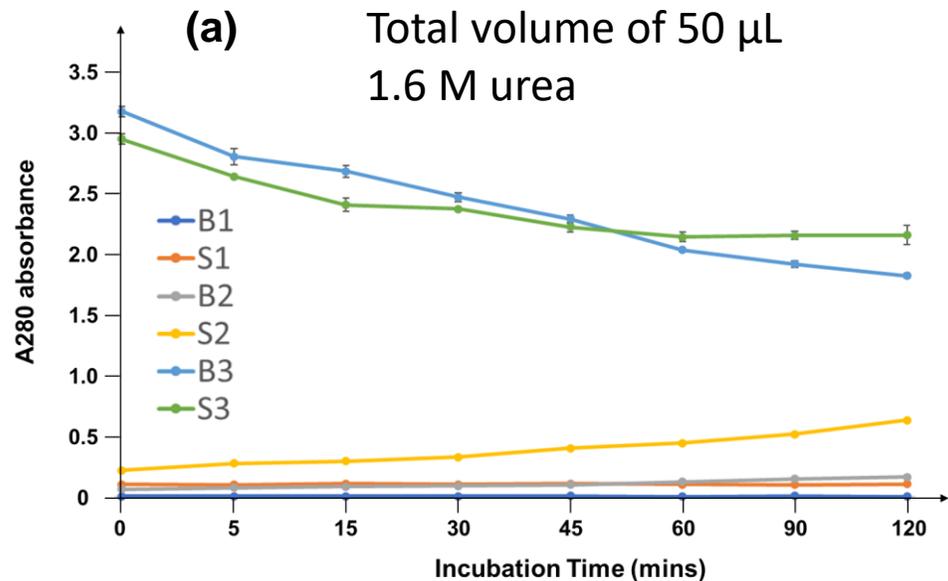


**Figure S1**

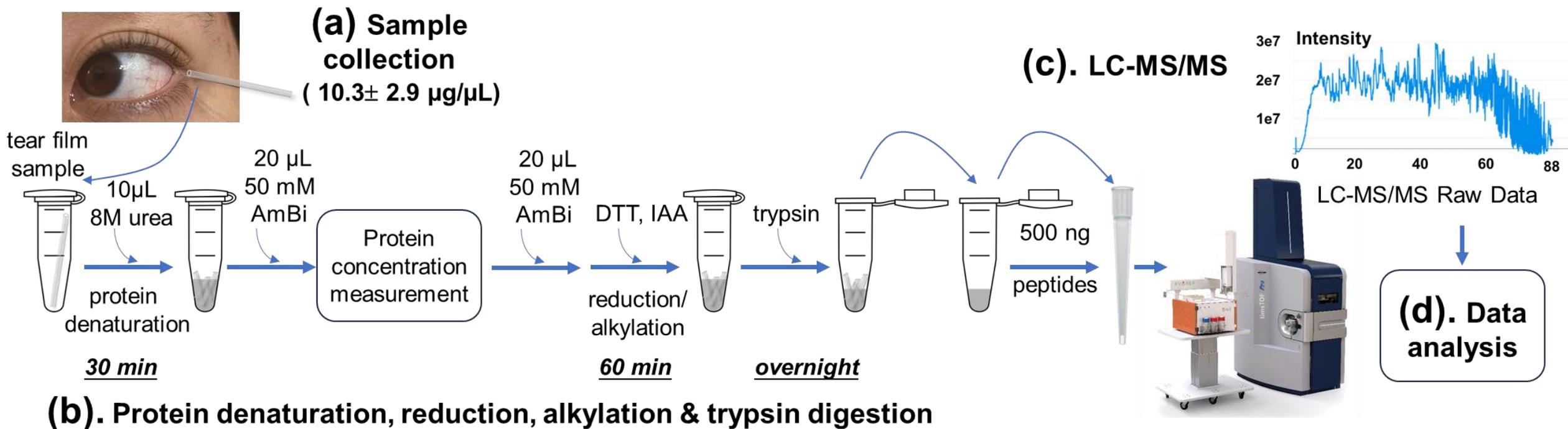


**B1.** Buffer without DTT & IAA  
**B2.** Buffer with 10 mM DTT  
**B3.** Buffer with 10 mM DTT, 25 mM IAA

**S1.** Tear sample without DTT & IAA  
**S2.** Tear sample with 10 mM DTT  
**S3.** Tear samples with 10 mM DTT, 25 mM IAA

**Figure S1. The effect of dithiothreitol (DTT) and iodoacetamide (IAA) on tear protein concentration measurements.** Pooled tear samples were aliquoted into capillary tubes, each containing 0.5  $\mu\text{L}$  of tear fluid, and placed in individual microcentrifuge tubes. To each tube, 10  $\mu\text{L}$  of 8 M urea solution was added, and the samples were incubated at room temperature for 30 minutes to denature the proteins. Next, either 40  $\mu\text{L}$  (**a & b**) or 20  $\mu\text{L}$  (**c & d**) of 50 mM ammonium bicarbonate solution was added to dilute the urea concentration. DTT and IAA were then added to the tubes as follows -- S1: No DTT or IAA added; S2: 10 mM DTT added; S3: 10 mM DTT added followed by 25 mM IAA. The volumes in all tubes were adjusted to be equal using 50 mM ammonium bicarbonate solution. Corresponding buffer controls (B1, B2, and B3) were prepared without tear proteins to match the experimental conditions of the tear samples. The tear protein and buffer solutions were incubated at room temperature, and the A280 absorbance was measured at 0, 5, 15, 30, 45, 60, 90, and 120 minutes using a NanoDrop spectrophotometer. **(a)** A280 absorbance readings for B1, B2, B3, S1, S2, and S3 were taken at various time points after 40  $\mu\text{L}$  of 50 mM ammonium bicarbonate was added, diluting the urea concentration from 8 M to 1.6 M prior to the addition of DTT and IAA, or an equivalent volume of 50 mM ammonium bicarbonate solution. **(b)** A280 absorbance readings for B1, B2, S1, and S2 are shown to visualize the data for the conditions described in (a). **(c)** A280 absorbance readings for B1, B2, B3, S1, S2, and S3 were taken at various time points after 20  $\mu\text{L}$  of 50 mM ammonium bicarbonate was added, diluting the urea concentration from 8 M to 2.67 M before the addition of DTT and IAA, or an equivalent volume of 50 mM ammonium bicarbonate solution. **(d)** A280 absorbance readings for B1, B2, S1, and S2 are shown to visualize the data for the conditions described in (c).

## Figure S2



**Figure S2. A novel in-capillary digestion workflow for enhanced tear proteomics in biomarker discovery.** This LC-MS/MS workflow was optimized for tear samples as small as 0.5  $\mu\text{L}$ , collected using glass capillary tubes. (a) Tear fluid was collected using 0.5  $\mu\text{L}$  glass capillary tubes, with an average protein concentration of  $10.3 \pm 2.9 \mu\text{g}/\mu\text{L}$ . (b) The experimental workflow included protein denaturation, reduction, alkylation, and trypsin digestion. The capillary tube containing the 0.5  $\mu\text{L}$  tear sample was transferred into a Protein LoBind microcentrifuge tube and stored at 4°C or on dry ice for transport to the laboratory. To the microcentrifuge tube, 50  $\mu\text{L}$  of 8 M urea solution was added without flushing out the tear sample from the capillary. The tube was vigorously vortexed and centrifuged at maximum speed ( $\sim 20,000 \times g$ ) in a benchtop microcentrifuge for three cycles, breaking the capillary tube into small fragments and thoroughly mixing the tear fluid with the urea solution. The sample was incubated at room temperature for 30 minutes to ensure protein denaturation. Following incubation, 20  $\mu\text{L}$  of 50 mM ammonium bicarbonate solution was added to dilute the urea concentration from 8 M to 2.67 M. Protein concentration was measured using A280 absorbance on a NanoDrop spectrophotometer. After quantification, additional 50 mM ammonium bicarbonate solution was added prior to DTT reduction, IAA alkylation, and trypsin digestion. Once digestion was complete and the pH adjusted to 2–3, 500 ng of digested peptides were directly loaded onto an Evosep tip for LC-MS/MS analysis (c), followed by data processing and analysis (d).