

PLASMINOGEN PURIFICATION

(Dec. 2001, Modified at Nov.2003)

1. Plasma preparation

- * Thaw outdated plasma (approx 1.5L for a 750ml Lysine-Sepharose column) o/n at 4°C.
- * Make plasma 0.1 mM in PMSF (dissolved in DMSO then add to plasma, proteinase inhibitor – prevents Pg degradation) and 10 mM in NaCitrate (prevents clotting).
- * Filter through glass wool, then through coarse filter paper.

2. Lysine Sepharose

- * Equilibrate column – approx 750 ml of 50 mM Tris, pH 7.4.
- * Load plasma onto Lys-Sepharose (at RT or 4°C, O/N in cold room is OK).
- * Remove non-specifically bound proteins: wash with 3–4 L of 50 mM Tris, 1 M NaCl, PH 7.4. – This step can be done overnight, but make sure not to dry out—make dead loop in tubing).
- * Elute bound Pg: When A280 is less than 0.05, begin elution – Elute bound Pg with 50 mM Tris containing 0.1 M e-amino caproic acid (EACA), pH 7.4. Monitor A280. When absorbance reaches 0.5, begin to collect Pg, keep collecting until A280 is less than 0.5.

3. Remove EACA from Pg

- * Dialyze extensively against 10–20 L containers of H₂O at 4°C, change at least 3 times.

4. Freeze and lyophilize

Transfer Pg to 50 ml tube; put the tube in dry ice. Make hole on the top before lyophilizing. – Lyophilizer (at Tarry 15-732 contact person: Tom at Tarry 14–750 Phone 3-0541).

5. Reconstitution

Purified Pg should be reconstituted in 20 mM NaOAC, pH 4.5. Store in –80°C. For longer term storage, directly store lyophilized Pg in –80°C.

6. Column storage

Strip with 4L of 20nM NaOAC, pH 4.0. Wash with 2 L 50 mM Tris, 0.01% NaN₃. Store the column at 4°C.

To determine plasminogen concentration::

Turn on UV light of Spectrophotometer (DU640 in room 8-730), find the program A280 or PGCONC in Custom Applications.

Take 990 ul of Hepes buffer (or water) into the reading vial, click “read blank”. Then add 10 ul of reconstituted Pg in the reading vial (1:100 diluted the sample). Click “read sample”.

The PGCONC program will automatically calculate the concentration in mg/ml based on:

Plasminogen MW is 92,000.

1mM of PG = 92 mg/ml.

Sample (mg/ml) / 92 (mg/ml) x 1000(uM)= sample uM

For example, 11/6/03 sample reading is 17.16 mg/ml:

$17.16 / 92 \times 1000 = 186 \text{ uM} \times 2.8(\text{ml of volume}) / 30 (\text{uM}) = 17.4 (\text{ml})$ – can make 17.4 ml of 30 uM of PG stock by add 14.6 ml of 20 mM NaOAC, PH 4.0.

* Beer's law:

$$A = \epsilon l c$$

ϵ extinction coeff (M)

l = path length (1 cm)

C = conc.

The extinction coefficient for a 1% solution of Pg is 16.7 (PG1% = 10 mg/ml) So, = 1.67 for a 1 mg/ml solution

Read A280 / 1.67 x dilution factor = mg/ml