Calculation of quantity of AQP2 in one inner medulla in moles

Take the number of moles per microgram of protein and multiply by the number of micrograms of total protein in the inner medulla.

a) Calculating normalized content (moles/microgram IM protein) e.g. from Figure 1:

AQP2 band intensity approximately equivalent to 0.55 - 0.85 microgram standard.

0.85 micrograms of peptide in moles is: 0.85 micrograms ÷ 4200 micrograms/micromole (MW of peptide standard)
$$= 2 \times 10^{-4} \text{ micromoles or } \underline{2 \times 10^{-10} \text{ moles}}$$

Microgram quantity of IM loaded for the total AQP2 immunoblot was 12.
Therefore the normalized content: 2×10^{-10} moles ÷ 12 microgram
$$= \underline{1.7 \times 10^{-11} \text{ moles per microgram IM protein}}$$

If the same calculation is performed using 0.55: normalized content:
$$= \underline{1.1 \times 10^{-11} \text{ moles per microgram IM protein}}$$

b) Total inner medullary content of protein (typical measurement of protein from whole IM, 220 g rat)
$$= \underline{500 \text{ microgram}}$$

c) AQP2 per IM $\quad = 1.7 \times 10^{-11}$ moles per microgram x 500 micrograms = 8.4nmoles
$\qquad\qquad\qquad = 1.1 \times 10^{-11}$ moles per microgram x 500 micrograms = 5.4nmoles

Thus, the quantity of AQP2 per rat inner medulla is estimated to be:
$$= \underline{\textit{5.4 – 8.4nmoles}}$$

Calculation of quantity of AQP2 per cell

Assuming that there are approximately 12,500 mm of IMCD in the inner medulla (from integration of data from (Knepper, Danielson et al. 1977)) and approximately 634 cells per mm (Kishore, Terris et al. 1996):
$$= \underline{7.5 \text{ million IMCD cells per inner medulla}}.$$

Assuming the estimated amount of AQP2 per IM: 5.4nmoles to 8.4nmoles

$(5.4 \times 10^{-9}$ moles AQP2) x $(6 \times 10^{23}$ copies per mole) ÷ 7.5×10^{6} cells
$$= \underline{4.4 \times 10^{8} \text{ copies per cell}}.$$

$(8.4 \times 10^{-9}$ moles AQP2) x $(6 \times 10^{23}$ copies per mole) ÷ 7.5×10^{6} cells
$$= \underline{6.7 \times 10^{8} \text{ copies per cell}}.$$

Thus: the number of AQP2 molecules per IMCD cell is estimated to be between 4.4 – 6.7 x10{8} copies per cell.

References:

Kishore, B. K., J. M. Terris, et al. (1996). "Quantitation of aquaporin-2 abundance in microdissected collecting ducts: axial distribution and control by AVP." Am J Physiol **271**(1 Pt 2): F62-70.
Knepper, M. A., R. A. Danielson, et al. (1977). "Quantitative analysis of renal medullary anatomy in rats and rabbits." Kidney Int **12**(5): 313-23.