B. Endoglycosidase H

Since N-linked carbohydrates are added exclusively in the ER lumen, glycosylation is a good assay for translocation. Two of its limitations are obvious: if your protein contains no N-linked carbohydrate addition sites (Asn-X-Ser/Thr) you cannot use this assay. Since not all N-linked sites are used, and since a given site can be found to be used in some but not all copies of a protein, failure to get glycosylated does not necessarily mean the domain was not translocated. On the other hand, if a domain is glycosylated, you can be essentially certain that it was translocated. Thus it is an assay that is very specific but not necessarily sensitive -- high possibility of false negatives. I typically immunoprecipitate first and endo H second rather than the reverse. Carry out immunoprecipitation as usual to the stage of washed protein A - beads ready to add SDS gel buffer. At this point, add 50 ul 0.1 M Na citrate pH 5.5, 0.1% SDS and vortex, then heat to 100øC for 2 minutes, spin out the beads and divide the supernate into two aliquots. To one aliquot add 0.25 ul (a tiny bit) of endoglycosidase H (concentration 1 unit/ml); to the other aliquot add nothing. Incubate both at 37øC for 6 to 12 hours. Then dry both down in a speed vac, and add SDS gel loading buffer as usual to process samples for PAGE.

WARNING: Unless you want to get tremendous variation in lane width, be sure to add 25 ul of citrate buffer to all samples for the gel, and dry them all down before adding SDS gel sample buffer, e.g. put all samples in the same salts and volume.

If you want to endo H digest total products, simply take an aliquot of total translation products (e.g. 1 or 2 ul), dilute into 50 ul of citrate buffer pH 5.5, heat, cool to room temp, digest with endo H at 37øC, speed vac to dryness and dissolve in SDS gel sample buffer.