The diffusion blot protocol

Use diffusion blotting - the way we used to do this was to use the cooling plate in the Multiphor apparatus heated to about 50 C and then place a backed gel (usually plastic backed) on to the cooling plate (using water or a light parafin oil underneath the plastic back to provide good thermal contact). The blotting membrane is then placed on the gel surface (the membrane having been activated and soaked in Towbin buffer) and then wetted filter paper (5 thick sheets) placed on top of the membrane (wetted also in Towbin buffer) and finally topped off with dry filter paper (x 5 again) (all the same size as the gel) and covered in parafilm with some kind of weight on top - we did a time course using rainbow markers to assess transfer effciency against time and think 50 minutes was optimal for our set up - you should check this yourself. The nice thing with this set up tis that diffusion blotting (by heating you will speed up the process and is called a thermal blot I think) is a lot less efficient than electroblotting (only about 30%) but this is great as your antibody does not need a lot of protein/ target to be able to detect it and then there is plenty of protein left behind in your gel so that you can post stain with silver and then the blot will exactly match the spot pattern as you have stabilised the gel size by using the plastic backing We used to love this method - maybe I should get an application note done ?

The plastic backing I talk of is very special and is called GelBond - we sell this but there is only one manufacturer of this product in the world called FMC and they charge a premium for the stuff