Some information on antigen biotinylation:

Most descriptions on biotinylation are usually based on having mg quantities of protein. Provided that the right equipment is used, it is possible to biotinylate also small amounts of protein without significant loss of material, e.g. amounts of around 50 µg of protein.

Different types of biotinylation reagents can be used; e.g. **water-soluble:**

- EZ-Link-Sulfo-NHS-LC-Biotin, catalogue no. 21335 from Pierce – www.piercenet.com
- ChromaLink BiotinLabelling Kit, catalogue no. B-9007-105K – www.solulink.com

And one that needs to be **dissolved in DMF**

- SureLINK Chromophoric Biotin labelling kit, catalogue no. 86-00-01 from KPL – www.kpl.com

The DMF soluble biotin has certain advantages.

Depending on the concentration of the protein one typically uses biotin: protein molar ratios from around 20:1 to 60:1. The lower ratio is used when having the protein at quite high concentration (i.e. 1mg/ml or above) and the higher ratios when the protein is at lower concentrations (the higher ratio will here increase the chance of binding).
The conjugation is made by simply mixing the two components and incubating at room temperature for 30-60 min.

After conjugation the sample is dialysed against a suitable buffer (e.g. PBS) using either a Slide-A-Lyser Mini Unit for volumes from 10 µl up to 200 µl or a Slide-A-Lyser Dialysis Cassette for volumes above this. These products are all available from Pierce and can be found at their website (www.piercenet.com), they are available in different sizes and with different molecular cut-offs. When biotinylating small amounts of antigen, the Mini units are preferably used.

After having dialysed the sample, it is possible to determine the level of biotinylation by analysing it with the so called HABA-avidin method (EZ-Biotin Quantitation kit, catalogue no. 28005 from Pierce). However, if using the DMF-soluble SureLINK biotin, this in itself contains a chromophoric group which can be measured at 354 nm and by looking at both 280 nm and 354 nm one can directly determine the number of biotin molecules per protein. These determinations are useful when one intends later to further optimize the test. Finally, sodium azide (final conc 0,05% v/v) or Kathon CG (final conc. 0,15% v/v), can be added for preservation purposes. If the labeled protein is unstable at low concentrations high grade BSA can be added to 0,1% (w/w).

The concentration of antigen to use in the test should best be established through titration, but our experience is that one can use significantly less of the antigen compared to when it is used for coating plates and we suggest that one initially tests it at a couple of different concentrations (e.g. 0.1 to 0.5 µg/ml).

For optimal results one should of course best try out different biotin:protein ratios to see how many biotins per molecule that are needed to obtain optimal results in the ELISpot.
If optimising is not possible due to lack of antigen amounts the above ratios are likely to give you a reasonable degree of biotinylation (i.e. somewhere between 1-5 biotins per protein molecule). This way one will have the chance to make a first evaluation and compare this method with the normal protocol. If working, it would probably be worthwhile doing some further optimizations.