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ANSWERS:**Q1 How do I send the gel?**

To ensure a safe arrival of your gel it has to be stabilized for the transport. We recommend to weld the gel into a plastic bag with a small amount of water to keep it wet (in case of sending more than one gel, please use one bag per gel). Fasten it with adjacency tape unto a solid cardboard and put another cardboard on top of it. It is important to make sure that this “sandwich” is packed tightly so that the gel can’t slip during transport.

Please always send the gel as a parcel, because gels send in an envelope could be damaged by means of an electric franking machine. We recommend shipment via an overnight service with delivery by 12 p.m. (noon). In this case no refrigeration is necessary. Sending samples over the weekend should be avoided due to indefinable storage conditions. Please make sure that your samples are delivered by Friday 12 p.m. latest.

Q2 Where do I send the gel(s)? Which information do you require for the sample?

Please send the gel(s) with a completely filled out and signed sample submission form to the address denoted on the submission form. We will be happy to send you the form upon request or simply download it from our website at www.protagen.de

Q3 How do I mark the spots on the gel?

To be able to analyze your sample, we require a clear annotation of your gel image. In case of submitting your gel image electronically (via email to ProtID@protagen.de), make sure to mark the spot positions. If you enclose a print-out or photocopy of your gel image, you can mark the spot position in handwritten form. Please make sure that all spots receive an unambiguous identifier (e.g. Spot 1, Spot 2 etc.)

Since all interesting spots of a gel image are marked, it is important that you enlist only those spots into the sample submission form, which actually should be analyzed.

Q4 How should I stain my gel?

To get optimal results we recommend staining proteins with colloidal Coomassie[®]¹ (e.g. Coomassie[®] Brilliant Blue G-250, SERVA DensiStain Blue G Staining Solution, Cat. No. 35078.01). This staining protocol offers a high detection sensitivity of the proteins in the gel and is at the same time compatible with the analysis by mass

¹ Coomassie is a registered trademark of ICI (Imperial Chemicals Industries Organics Inc.)

spectrometry (MS). The staining protocol with Coomassie[®] Brilliant Blue R-250 (e.g. SERVA Blue R Tablet Staining Kit, Cat. No. 35079.01) is MS compatible as well but has a poorer sensitivity.

A protocol describing the staining process as well as literary references can be found at: www.serva.de/products/knowledge/041074.shtml

Q5 Can I use silver staining?

Yes, you can use MS compatible silver staining (e.g. SERVA Silver Staining Kit SDS PAGE, Cat. No. 35076.01). A protocol is described at Blum, H, Beier, H and Gross HJ. "*Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels*". Electrophoresis, 1987, 8: 93-99.

Please notice that you cannot use silver staining with e.g. glutaraldehyde. However the best identification results can be reached by staining with colloidal Coomassie[®] (see Q4).

Q6 How much sample must be applied to the gel?

Generally it can be said that the more protein you provide on the gel the better are the chances for successful protein identification. An amount of protein, which can be stained in the gel with Coomassie[®] or colloidal Coomassie[®] is normally enough to successfully identify the protein.

Q7 How old can the gel be?

After a longer storage period we observed a decline of the identification rate of the proteins in the gel. To prevent this, we recommend storing the gel not exceeding one week at 7°C.

Q8 Can you pool several bands from a protein to get more protein?

Generally the pooling of several gel pieces does not lead to better protein identification. Only an improved focusing of the bands or spots, meaning a higher local concentration of protein in the gel, enables better protein identification.

Q9 How well separated must the protein be? Can several proteins be identified from one band/spot?

A good separation is a prerequisite for successful protein identification via MALDI-MS, especially in the case of 1-D gel electrophoresis. Nevertheless, in some cases it is possible to identify several proteins in one spot/band. Because of the heterogeneity of the sample in some cases it may also happen that no protein can be identified.

Q10 Why should I send the whole gel and not excised spots?

For a high significance and success rate of protein identification, we have developed highly sensitive protocols and processes. They already comprise the picking of an optimal size of the gel piece as well as a careful avoidance of contamination, such as human keratin or cross-contamination. Therefore we recommend the sending of the intact gel.

Q11 Can samples from an organism be identified, whose genome is not yet sequenced?

The performed protein identification is based on a comparison of the measured data to already known proteins, which are registered in different protein databases. Due to this fact, only proteins which have already been characterized can be analyzed with this method.

However, the sequence of the presented protein must not fully correspond to the one given in the sequence database. Therefore the process is not restricted to organisms, from which the sequences originated from.

Q12 Can post-translational modifications be detected?

Within the Protein Identification Service described here, the detection of post-translational modification (like e.g. phosphorylation, acetylation etc.) is not possible. However, Protagen AG is well experienced in this type of analyses. We will be happy to discuss further details on this type of analyses with you. Please contact us directly for inquiries or questions.

Q13 Do you run MS/MS analysis?

Within the Protein Identification Service it is standard to not only run a peptide mass fingerprint (PMF) but also a peptide fragmentation fingerprint (PFF, MS/MS) analysis, presumed the amount of sample will be sufficient. With these fragment spectra the amino acid sequence of individual peptides can be determined. These data increases the significance of the results tremendously.

Q14 Which rate of success can be expected?

This is a question not easily answered, because the success of the analysis depends on many factors. The most important factor usually is the amount of protein available for analysis (see Q6). Other factors are the resolution of the protein spot/ band (see Q9), possible contaminations (see Q10), availability in the protein database (see Q11), age of the gel (see Q7), size of the protein and the primary sequence of the protein. For a well focused 2-D gel spot with a medium staining intensity

(Coomassie[®]) and a molecular weight above 15kDa from an already sequenced genome, a probability of success of over 95% can be expected.

Q15 How and when do I get the results?

The results will be summarized in a report (= Protein Information Summary), which contains all details of the protein analysis such as the protein sequence or the identified protein, the identified peptides and the sequence coverage. For all details please take a look at an example of such a Protein Information Summary at our website at www.protagen.de.

Timeline: Normally we are able to send you the results via email within the first week (5 working days) upon submitting of the gel(s). In any case you will receive your results not later than two weeks after we have received your samples.

Q16 What do I need to publish my work?

If the analysis results are intended for publication, you can refer to the following publication of Protagen AG, which describes the process in detail (Lutter P, Meyer HE, Langer M, Witthohn K, Dormeyer W, Sickmann A, Blueggel M, Electrophoresis, 2001 Aug; 22 (14): 2888-97).

If you need further details for your material and methods section, please contact us via email (ProtID@protagen.de). Please note, that we generally do not provide mass spectra.

Q17 What is Gelviewer Pack&Go and how can I use it?

Gelviewer Pack&Go is a stand-alone software package derived from the data analysis software ProteinScape™ (Bruker Daltonik GmbH/Protagen AG) used for protein identification.

It is a fully functional electronic report which enables you to use all its intrinsic advantages, as for example linkage of the protein identification result to the spot position on the gel image (navigating by gel) and further external links, e.g. NCBI entry. You can also use this format for generating your own reports/publications by creating and embedding figures.

Note: Before using Gelviewer Pack&Go, please download the file from your GPCF website and install the software once. We can only create a Gelviewer Pack&Go file for your analysis if we received your gel image by email.

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