Approximately $4*10^6$-$4*10^{10}$ cell will be needed and about 300-500 ug total protein is required for one gel for protein ID purposes.

1). Pellet the cells in a suitable centrifuge at 4C.
2). Pour off all growth media, taking care not to disturb the cell pellet,
3). Re-suspend the cell pellet in 1ml of standard cell wash buffer in a microfuge tube.
4). Pellet the cells in a bench-top microfuge at 1200o x g for 4 min at 4C.
5). Remove and discard the supernatant.
6). Re-suspend the cell pellet in 1 ml standard cell wash buffer in a microfuge tube.
7). Repeat step 4-6 at least 3 times.
8). Ensure all the standard cell wash buffer has been removed.
9). Re-suspend the washed cell pellet in 1ml of standard cell lysis buffer and leave on ice for 10 min.

If the protein concentration if less than 5mg/ml after protein quantitation, re-suspend cells in a smaller volume of lysis buffer in subsequent experiments. Alternatively, precipitate proteins using Ettan 2-D clean-up kit (code number 80-6484-51) and re-suspend in a small volume of standard cell lysis buffer.

10). Keep the cells on ice and sonicate in intermittently until the cells are lysed.
11). Centrifuge the cell lysate at 4C for 10 min at 12000 x g in a microcentrifuge.
12). Transfer supernatant to a labeled tube. This is the cell lysate. Discard the pellet.
13). Check the pH of the cell lysate is still at pH 8.0-9.0 by spotting 3 ul on a pH indicator strip. If the pH of the cell lysate has fallen below pH 8.0 then the pH of the lysate will need to be adjusted before labeling. (Again, we might not need this adjustment).
14). The cell lysate can now be store in aliquots at -70C until protein yield is to be determined.
15). Kits can be used to remove some of the abundant proteins (ie albumin depletion kits) or fractionate

Tables:

1. Standard cell wash buffer

Reagent	Quantity	Final Concentration
Tris (100mM, pH8.0)	5.0 ml	10 mM
Magnesium acetate (1 M)	0.25 ml	5 mM
Make up to 50 ml with distilled water		

2. Standard cell lysis buffer

Option 1

Reagent	Quantity	Final Concentration
Tris (1M, not pH'd)	3.0 ml	30 mM

Thiourea (MW 76.12)	15.22 g	2 M
Urea (MW 60.06)	42.0 g	7 M
CHAPS (MW 614.89)	4.0 g	4% (w/v)
Diluted HCl	-	-
Make up to 100 ml with distilled water		

Adjust to pH 8.5 with diluted HCl. Small aliquots can be stored at -20C, for up to 3 months.

Option 2

Reagent	Quantity	Final Concentration
Tris (1M, not pH'd)	3.0 ml	30 mM
Urea (MW 60.06)	42.0 g	7 M
CHAPS (MW 614.89)	4.0 g	4% (w/v)
Diluted HCl	-	-
Make up to 100 ml with distilled water		

Adjust to pH 8.5 with diluted HCl. Small aliquots can be stored at -20C, for up to 3 months.

Notes
Requirements for a cell wash buffer

This section assumes that the biological material under analysis is a cell culture.
The requirements for a cell wash buffer are that it should not lyse the cell, but it should dilute and remove any growth media, or reagents that might affect the CyDye DIGE Fluor minimal dye labeling process.

1. Requirements for a cell lysis buffer

Ensure that the pH remains between pH 8.0-9.0, by including a buffer such as Tris, HEPES or Bicarbonate in the protein solution. The buffer should be at a concentration of approximately 30 mM. Higher buffer concentrations may affect isoelectric focusing. Failure to include a suitable buffer will mean that the pH of the solution may fall below pH8.0 resulting in little or no protein labeling, The standard cell lysis buffer is required to work at 4C so that pH should be checked when the solution was chilled.