Optimization of loading conditions on Capto adhere using design of experiments

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Optimization of loading conditions on Capto adhere using design of experiments

Summary

Capto™ adhere is a strong ion exchanger with multimodal functionality designed for intermediate purification and polishing of monoclonal antibodies. Removal of remaining contaminants is achieved in flowthrough mode under conditions that allow the antibodies to pass directly through the column while the contaminants are adsorbed.

This Application Note describes the optimization of the loading conditions to obtain the window of operation for Capto adhere. In order to find the optimal conditions, a full factorial design of experiment (DoE) was used with three variables: pH, conductivity, and load. The implications of each result are discussed and general trends for how pH, conductivity, and sample load affect yield and purity are outlined.

The results demonstrate that it is possible to find a wide window of operation in terms of pH and conductivity.

Introduction

Capto adhere is a multimodal ion exchanger designed for intermediate purification and polishing of monoclonal antibodies (MAbs) after a capture step on Protein A medium (Fig 1).

The multimodal functionality gives a different selectivity compared to traditional anion exchangers. Removal of leached protein A, antibody dimers and aggregates, host cell proteins (HCP), viruses, and nucleic acids can be performed in flowthrough mode where the antibodies pass directly through the column while the contaminants are adsorbed. Capto adhere improves yield, productivity, and process economy with:

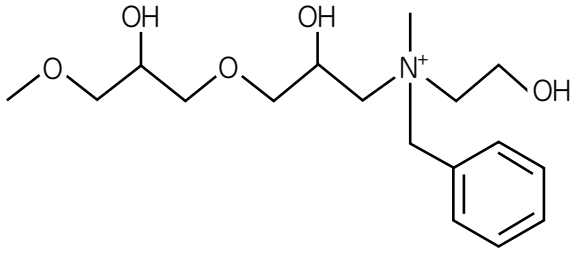

Fig 1. The Capto adhere ligand, N-benzyl-N-methyl ethanolamine, exhibits many functionalities for interaction. The most pronounced are ionic interaction, hydrogen bonding, and hydrophobic interaction.

- High capacity and productivity
- Contaminant removal to formulation levels in one step
- Wider operational window of pH and conductivity
- Savings in time and operating costs with a two step chromatographic process

As a member of the BioProcess™ media family, Capto adhere meets the demands of industrial biotechnology with validated manufacturing methods, security of supply, and comprehensive regulatory support to assist process development, validation, and submission to regulatory authorities.

Design of experiments (DoE) – basic principles

DoE is a systematic approach to investigate how variations in factors (X's) affect the responses (Y's) in a system (e.g., determining the mathematical relationship between X and Y). DoE is used to plan experiments so that the maximum amount of information can be extracted from the performed experiments. The factors in a DoE study are simultaneously varied so that they are independent of each other in a statistical sense. This makes it possible to evaluate the effect on the response of each factor separately (main effects). In addition, interaction effects between factors can be evaluated. For optimizing purposes, the use of DoE greatly increases the likelihood that the real optimum for a response is found.

A commonly used type of DoE is full factorial design, which is used both for screening and optimization purposes. A great advantage with the full factorial design is that all main effects and interaction effects are independent of each other and therefore their effect on the response can be resolved in the evaluation. A disadvantage with the full factorial design is that the number of experiments increases as the number of factors studied increases - the number of experiments is 2^n where n is the number of factors. A full factorial design with seven factors would need $2^7 = 256$ experiments. When many factors are included in the design, there are other types of DoE that can be used, which will significantly reduce the number of experiments, with the trade-off that some information is lost.

Center points are important for the DoE. The center point is usually replicated and will give information on the variation in the responses. The center points will also provide information on possible curvature in the data.

Method design and optimization

Balancing product yield against product purity is the major consideration when optimizing a method. When running in flowthrough mode, loading conditions will usually be a compromise between conditions favoring yield and conditions favoring contaminant clearance. By adjusting pH and conductivity of the sample as well as the sample load, conditions can be obtained where most contaminants are adsorbed while the monomeric antibodies pass through the column. Optimization of loading conditions is preferably performed by using DoE. A common approach in DoE is to define a reference experiment (center point) and perform representative experiments around that point. To be able to define the center point and the variable ranges, some initial experiments are required.

Establish non-binding conditions

To find conditions suitable for the DoE, initial experiments can be performed in binding mode, using a pH gradient for elution (Fig 2). The elution position (i.e., pH at peak maximum) defines the lower pH in the design. The upper pH in the design should normally be about two pH units higher. Experiments can also be performed in flowthrough mode, keeping the conductivity constant at a moderate level. A comparison of chromatograms is shown in Figure 3. At high pH (i.e., close to pI for the antibodies) the breakthrough during sample load is delayed, the breakthrough and wash curves are shallow, and significant amounts of MAb binds to the adsorbent. A decrease in pH (i.e., further from pI) results in weaker electrostatic interaction between the antibodies and the adsorbent, steeper breakthrough and wash curves, and increased yield.

Column: Tricorn™ 5/100 packed with 2 ml Capto adhere; bed height 10.5 cm
Sample: Feed containing monoclonal IgG_1, rProtein A elution pool, desalted
Sample load: 1 mg IgG_1/ml medium
Buffer A: 20 mM sodium citrate + 20 mM sodium phosphate pH 7.8
Buffer B: 20 mM sodium citrate + 20 mM sodium phosphate pH 4.0
Flow velocity: 200 cm/h
System: ÄKTAexplorer™ 100

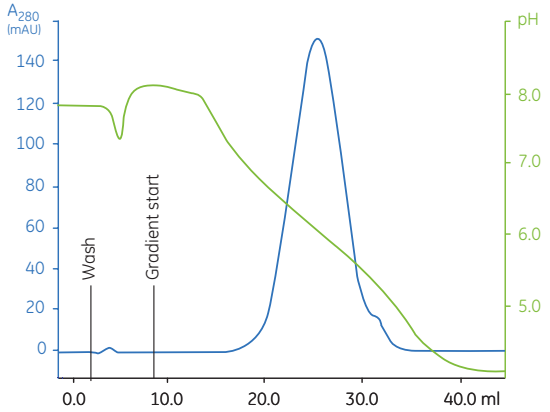

Fig 2. Establishing suitable conditions for DoE on Capto adhere in binding mode.

Column: Tricorn 5/20 packed with 0.5 ml Capto adhere; bed height 2.5 cm
Sample: Feed containing monoclonal IgG_1, rProtein A elution pool, desalted
Sample load: 75 mg IgG_1/ml medium
Loading buffer: 25mM Bis-Tris, pH 6.0 or 35mM Tris, pH 8.0
Elution buffer: 100 mM acetic acid pH 4.0
Flow rate: 0.25 ml/min (2 min residence time)
System: ÄKTAexplorer 10

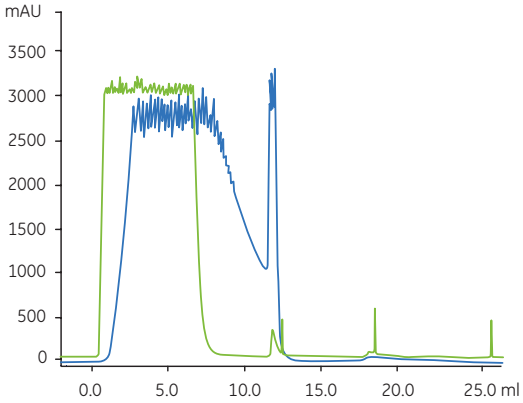

Fig 3. Establishing suitable conditions for DoE on Capto adhere in flowthrough mode. Comparison of chromatograms obtained at different pH: pH 8.0 (blue curve) and pH 6.0 (green curve).

In the DoE, pH, conductivity, and load must be included. It is important to include conditions at the higher pH range resulting in lower yield and higher purity, as well as conditions at lower pH range resulting in higher yield and lower purity.

Setup of a full factorial DoE with three parameters

Below is a stepwise description of how to set up a full factorial design.

1. **Work prior to actual setup of the design**

   Perform initial loading experiments at varying pH, as described above. Choose parameters to include and define parameter ranges and responses.

2. **Choose design for screening or optimization**

   Full factorial design is commonly used in both screening and optimization. A full factorial DoE in three parameters will give $2^3 = 8$ experiments + center points. A graphical view of how the experiments are organized is shown in Figure 4.

3. **Choose center points for the design**

   Center points are important in DoE because they give an indication if there is curvature in the data. Replicated center points are recommended. For example, a full factorial design in three parameters with three center points gives a total of 11 experiments.

4. **Systematic variation of the parameters**

   Limiting values, high and low, should be used for each parameter. The high and low values should be combined in a way that makes the parameters independent (to be able to separate effects).

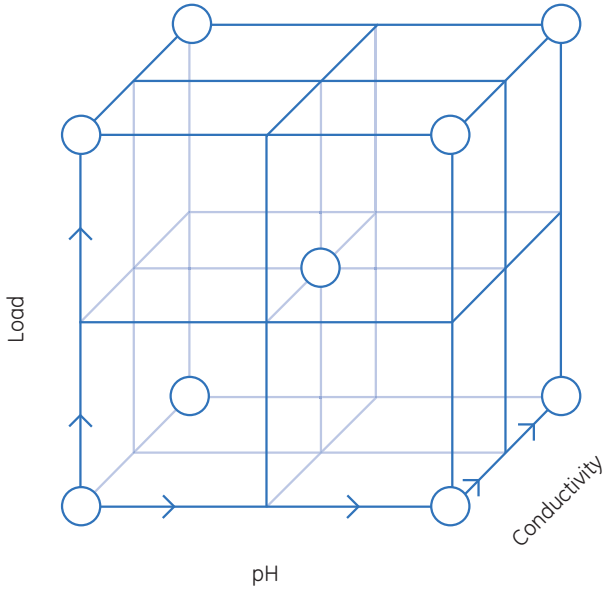

Fig 4. Graphical representation of a full factorial design in three variables with center points.

DoE used for purification of an IgG_1 MAb

DoE was applied for the optimization of loading conditions for an antibody, previously purified on non-agarose based rProtein A chromatographic medium. The experiments were designed and evaluated using Umetrics Modde™ 7.0 software (www.umetrics.com).

The feed contains a monoclonal IgG_1 expressed in CHO cell supernatant with pI about 9. The impurity levels after Protein A were determined: leached protein A 36 ppm; dimers and aggregates 3.3%, and HCP 210 ppm. The experimental setup was a full factorial design with three variables: load, pH (based on Figs 2 and 3), and conductivity, with additional points to resolve curvature effects (Table 1). In total, 14 experiments were included in the model, and the measured responses were yield and concentration of impurities [Protein A (ppm), dimers and aggregates (%), and HCP (ppm)] in the flowthrough pool. For each response a separate model was calculated. The models were fitted to MLR (multiple linear regression) and are well explained and show good stability to cross validation. Response surfaces were obtained for yield as well as for clearance of key contaminants.

Table 1. Design setup, includes two center points (bold) and four additional points at pH 7 to resolve curvature effects

Load (mg mAb/ml)	pH	Cond (mS/cm)
75	6	2
300	6	2
75	8	2
300	8	2
75	6	15
300	6	15
75	8	15
300	8	15
187.5	**7**	**8.5**
187.5	**7**	**8.5**
75	7	15
300	7	15
187.5	7	2
187.5	7	15

Results

Parameters affecting the yield

The parameters that affect the yield are shown in the coefficient plot[1] (Fig 5). The plot shows that high sample load, low pH, and high conductivity results in high yield. The interaction effects (load × pH , load × conductivity) are also significant for the yield response. The response surfaces (Fig 6) show that higher loads will give a larger pH window with yield > 90%.

[1] The coefficient plot describes the impact of investigated parameters on the yield. In this experiment, load is positively correlated to the yield, implying that a higher load will give a higher yield; pH is negatively correlated to the yield, meaning that a lower pH will give a higher yield, and conductivity is positively correlated to yield, but to a smaller extent, meaning that a higher conductivity will give higher yield.

The interaction effects that are present in the coefficient plot (load × pH, load × conductivity) mean that if pH is changed, the yield will not only change with the effect of pH but also with the effect of load at that specific pH. The same discussion can be applied to the load × conductivity interaction effect.

Parameters affecting the Protein A clearance

The coefficient plot shows that a high pH will give good Protein A clearance (Fig 7). The conductivity alone does not affect the response, but there is a significant interaction effect for pH × conductivity. If this term is high, the Protein A clearance will be low. Load was not a significant factor for this response.

The response surfaces (Fig 8) show that high pH and low conductivity will give high Protein A clearance.

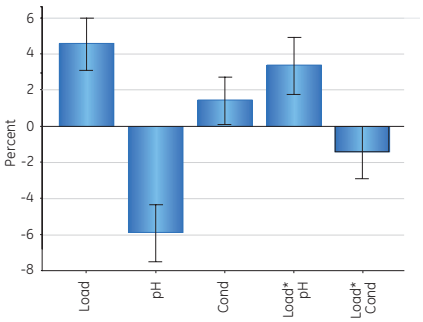

Fig 5. Coefficient plot for the yield model.

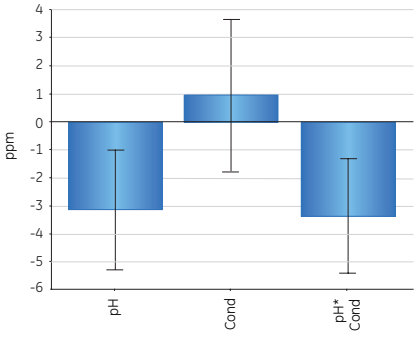

Fig 7. Coefficient plot for the Protein A clearance model.

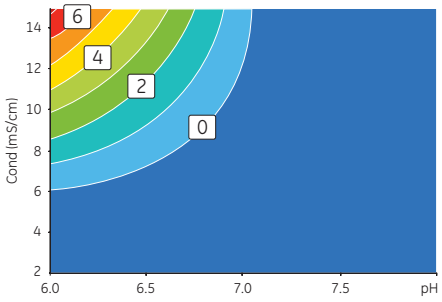

Fig 8. Response surfaces for the Protein A clearance model, conductivity versus pH. Protein A concentration expressed in ppm.

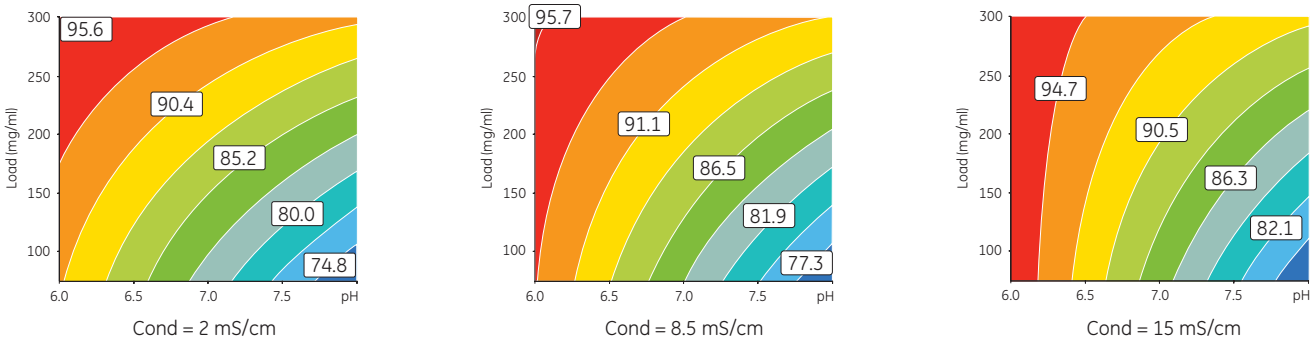

Fig 6. Response surfaces for the yield model. Load versus pH at different conductivities, with yield expressed in percent (labels).

Parameters affecting dimers and aggregates clearance

The coefficient plot shows that pH is the most important parameter and that high pH will give a high dimers and aggregates clearance in the flowthrough pool (Fig 9). The load parameter is also significant, but very small. The load should be low to give high dimers and aggregates clearance. There is a significant curvature effect assigned to pH. If pH is too high or too low, the aggregates clearance will be less efficient. The conductivity did not significantly affect dimers and aggregates clearance.

The response curve (Fig 10) shows that the load has only a small effect on aggregates clearance, so only pH needs to be considered.

Parameters affecting host cell protein (HCP) clearance

The coefficient plot (Fig 11) and response curves (Fig 12) show that low sample load, low conductivity, and high pH will give the best HCP clearance.

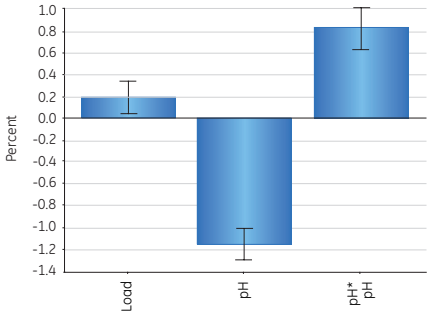

Fig 9. Coefficient plot for the dimers and aggregates clearance model.

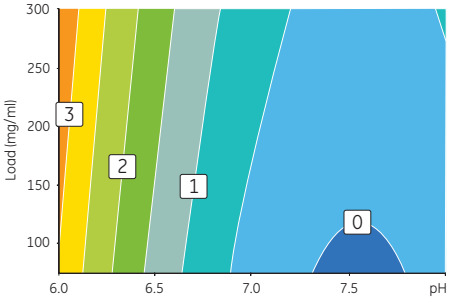

Fig 10. Response curve for the dimers and aggregates clearance model, load versus pH. Dimers and aggregates concentration expressed in percent.

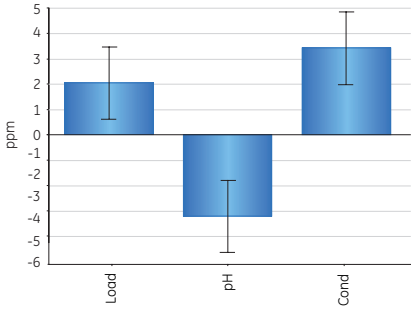

Fig 11. Coefficient plot for the HCP clearance model.

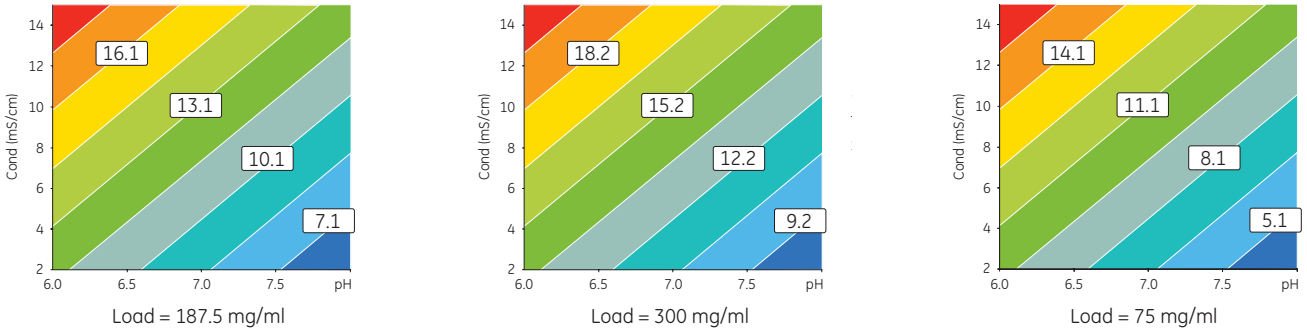

Fig 12. Response surfaces for the HCP clearance model, conductivity versus pH at different loads. HCP concentration is expressed in ppm.

Conclusions - optimal loading conditions and general trends

Each monoclonal antibody is unique and the level of contaminants varies between different cell lines and differences in previous purification steps. This implies that it may be difficult to predict optimal loading conditions. However, based on design of experiments performed with several different antibodies, some general trends have been identified (Fig 13).

- For best yield load should be high, the pH low, and conductivity high.
- For the best dimers and aggregates clearance, the pH should be high, while load and conductivity should be low. Dimers and aggregates clearance is typically less affected by conductivity than Protein A and HCP clearance.
- For the best Protein A and HCP clearance, the pH should be high and conductivity low.

Loading conditions will therefore be a compromise between conditions favoring yield and conditions favoring contaminant clearance. Optimal loading conditions will be a balance between load, pH, and conductivity. Consequently, for optimization of the loading step, all three parameters should be varied in the same experimental series.

Optimal loading conditions for five MAbs together with yield and contaminant clearance results from two step process, including protein A medium and Capto adhere, are shown in Table 2. pH should normally be well below the isoelectric point, while optimal conductivity is harder to predict.

The response surfaces above show the influence of sample load, pH, and conductivity on four different responses (yield of monomeric MAb and clearance of Protein A, dimers and aggregates, and HCP, respectively), and how to reach desired values for each of them. Even though the optimal conditions for each response are not the same, there is a large area where acceptable values can be obtained for all four responses. Suggested loading conditions for this MAb when purified with Capto adhere are a sample load of 200 mg/ml, pH 7, and conductivity 8.5 mS/cm. The expected outcome would be a yield of over 90%, leached Protein A below the detection limit, dimers and aggregates < 0.5%, and HCP concentration of < 15 ppm.

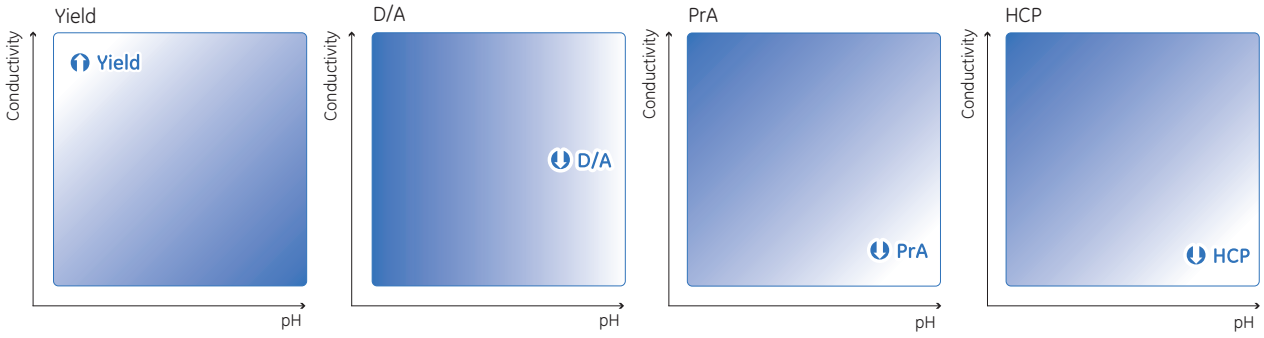

Fig 13. General trends with respect to loading conditions for yield, dimers and aggregates, and Protein A and HCP clearance.

Table 2. Optimal loading conditions for different MAbs with regard to yield and clearance of HCP, Protein A, and dimers and aggregates

MAb	pI	pH	Conductivity (mS/cm)	Yield %	D/A %	Protein A ppm	HCP ppm
1	~ 9	7	8	90	0.5	n.q.	< 15
2	8.3 to 8.9	5.5	3	95	0.6	n.q.	2
3	7.5 to 8.4	6	2	95	0.8	n.q.	9
4	7.7 to 8.0	7	20	91	0.2	n.q.	30
5	6.5 to 9.0	7.5	20	92	< 0.1	n.q.	7.5

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Ltd
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway,
NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku,
Tokyo 169-0073, Japan

Asia Pacific T +85 65 62751830 F +85 65 62751829 • Australasia T +61 2 8820 8299 F +61 2 8820 8200 • Austria T 01 /57606 1613 F 01 /57606 1614 • Belgium T 0800 73 890 F 02 416 8206 • Canada T 1 800 463 5800 F 1 800 567 1008 • Central & East Europe T +43 1 972 720 F +43 1 972 722 750 • Denmark T +45 70 25 24 50 F +45 45 16 2424 • Eire T 1 800 709992 F +44 1494 542010 • Finland & Baltics T +358 9 512 3940 F +358 9 512 39439 • France T 01 69 35 67 00 F 01 69 41 98 77 Germany T 0800 9080 711 F 0800 9080 712 • Greater China T +852 2100 6300 F +852 2100 6338 • Italy T 02 26001 320 F 02 26001 399 • Japan T 81 3 5331 9336 F 81 3 5331 9370 • Korea T 82 2 6201 3700 F 82 2 6201 3803 • Latin America T +55 11 3933 7300 F +55 11 3933 7304 • Middle East & Africa T +30 210 96 00 687 F +30 210 96 00 693 • Netherlands T 0800-82 82 82 1 F 0800-82 82 82 4 • Norway T +47 815 65 777 F +47 815 65 666 • Portugal T 21 417 7035 F 21 417 3184 • Russia, CIS & NIS T +7 495 956 5177 F +7 495 956 5176 • Spain T 902 11 72 65 F 935 94 49 65 • Sweden T 018 612 1900 F 018 612 1910 • Switzerland T 0848 8028 10 F 0848 8028 11 • UK T 0800 515 313 F 0800 616 927 • USA T +1 800 526 3593 F +1 877 295 8102

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