Monoclonal antibody disulfide reduction during manufacturing
Untangling process effects from product effects

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**Introduction**

The target specificity, favorable pharmacokinetics and pharmacodynamics, and stability of monoclonal human immunoglobulin gamma (IgG) antibodies have resulted in their widespread use in the biopharmaceutical industry.1,2 Commercial therapeutic antibody production is a complex but fairly well established process, typically involving expression in Chinese hamster ovary cells (CHO), harvesting of the secreted protein, and a series of chromatography steps to remove impurities. Reduction of antibody interchain disulfide bonds during manufacturing operations has recently been the subject of much interest.3-5 This phenomenon is observed when extending the time that the antibody remains in the cell culture fluid (CCF) or harvested cell culture fluid (HCCF) in the “harvest” step of production. This harvest step includes separation of cells from the media prior to the first column purification.

Process-induced antibody disulfide bond reduction has been observed inconsistently at large scale processes and is not typically observed with standard bench-scale (up to 10 L) models.2 This reduction has been attributed to certain enzymes that are released from the intracellular compartments of lysed cells. Components in the thioredoxin reduction pathway, including thioredoxin reductase and NADPH, have been proposed as the principal underlying contributor for this antibody disulfide bond reduction.3,4 Reduction has been shown to be virtually eliminated by maintaining dissolved oxygen (DO) levels during harvest operations.5 In addition, the cysteine/cystine redox couple, which is present in the growth media, may affect disulfide bond formation, reduction, and rearrangement.6 Likewise, many other media components, such as certain metal ions and their complexes, are likely to affect the reduction potential during the harvest procedure.5,6

In these studies, cell lysis and an anaerobic environment both promoted antibody reduction during harvest;5,6 therefore,
were observed. Because cell cultures, cell lines, and the products themselves can vary in cell cultures expressing two different antibody products, the underlying causes for these reduction differences could not be determined. The study presented here explores the relationship between reduction and process variables, separating the influence of process and products to demonstrate that CHO cell line or cell culture process can dramatically influence reduction during harvest operations and that the antibody class and light chain type also influence the extent of that reduction.

**Results**

**Small scale model.** Harvest-related disulfide reduction has been reported as highly dependent on process scale and has been reported in some scaled-up, but not bench-scale, processes. This effect of scale may be attributed to the maintenance of oxygen in small-scale harvests, which may preserve disulfide bonds. Typically, bench-scale experiments are open to the air, which allows more efficient oxygen transfer than typical manufacturing-scale (15,000 to 20,000 L) cell culture production. Bench-scale experiments also use different centrifuge equipment, introducing the possibility of different degrees of cell shearing during removal of debris. To facilitate harvest reduction experiments, a small-scale model, similar to previously described models, was developed. A “worst case” reduction model of cell culture extract was generated by mechanically shearing 2 L of whole cell culture fluid (CCF) used for production of an IgG1κ mAb (mAb A), transferring the sheared CCF into a 3 L bioreactor, and sparging the resultant slurry with nitrogen to simulate the anaerobic environment of the commercial scales. Samples were taken at 0, 0.5, 1, 2, 4, 8, and 24 h and immediately frozen at −70°C. Non-reduced capillary electrophoresis with sodium dodecyl sulfate (NR CE-SDS) was performed on all samples to measure the degree of interchain disulfide bond breakage. Representative electropherograms of a partially reduced antibody, a properly disulfide-linked antibody, and a blank are shown in Figure 1. This figure shows that the peaks in the pre-peak region of the electropherogram increase in intensity relative to the main, properly disulfide-linked, peak. These pre-peaks have been shown to be light chain (L), heavy chain (H), and combinations of the two chains (HL, HH, HHL). Because size exclusion chromatography indicates that reduction does not result in disassembly of the antibody chains, the NR CE-SDS pre-peaks represent properly assembled antibodies with one or more broken interchain disulfide bonds. The relative area associated with the pre-peaks and main peak were used to monitor interchain disulfide reduction in a series of harvest experiments. Results showing antibody reduction in mAb A for up to 24 h after cell shearing in the small-scale model are shown in Figure 2. An increase in the percentage of pre-peaks over time is observed, from 9% at the initial time point to ~45% at 8 h. This increase in the percentage of pre-peaks replicates previously published results and demonstrates that the small-scale model is capable of inducing and monitoring disulfide bond reduction. It is worth noting that the pre-peak level decreases after 8 h, and it is only ~12% by 24 h, indicating that disulfide bonds can reform. This observation is consistent with previously published results.

**Product, cell line and process.** Partial disulfide bond reduction behavior was probed with multiple Amgen therapeutic antibodies and cell lines. Three products, an IgG2α (mAb B), an IgG2κ (mAb C), and mAb A, the IgG1κ discussed above, were tested by shearing end of production cells and subjecting the lysed CCF to nitrogen sparging in the small-scale model at 25°C. Figure 3 displays the relative amount of intact antibody for each of these products as a function of time. Although the IgG1κ results demonstrate that an interchain disulfide can be reduced in this antibody type using this small-scale model, no changes were seen in mAb C (IgG2κ) or mAb B (IgG2α). This lack of reduction under these reduction promoting conditions has not been previously reported, and indicates tight controls of air sparging.
or cell shearing are not necessary for all antibody production processes. This comparison of end of production CCF shows stark differences in behavior, but does not distinguish between the effects of product, cell line, or cell culture process. While the mAb C (IgG2κ) titer and cell density are fairly low, and that might account for the difference, both the mAb A (IgG1κ) and the mAb B (IgG2α) have relatively high titers and cell densities, as shown in Table 1. IgG2s, such as the mAb B, are known to be less susceptible to reduction by thioredoxin; however, there could also be differences between the cell lines or processes that could contribute to these observations.

Direct comparison between cell lines is complicated due to the differences that may arise through the transfection process. Both copy number and insertion site can vary from clone to clone, and both of these parameters may also affect cell growth, viability, productivity, and metabolism. Therefore, to partially disentangle the effect of product, cell line and process on the level of reduction, end of production cells from these three products were lysed, and the original product was removed via Protein A affinity to create soluble cellular component material. The reduction activity was shown to be maintained through this type of processing by Trexler-Schmidt et al. The results in Table 2 illustrate the difference in NR CE-SDS % Main peak between the t0 and 8 h samples for several combinations of cells and purified products. To determine whether this material remained active, purified mAb A was spiked back into its own soluble cellular components and held under nitrogen overlay for 8 h. When mAb A sheared cell broth was reconstituted in this manner, the difference in NR CE-SDS % Main peak between the two of these products was ~45%, identical to the product activity was shown to be maintained through this type of processing by Trexler-Schmidt et al. The results in Table 2 illustrate the difference in NR CE-SDS % Main peak between the t0 and 8 h samples for several combinations of cells and purified products. To determine whether this material retained activity, purified mAb A was spiked into its own soluble cellular components and held under nitrogen overlay for 8 h. When mAb A sheared cell broth was reconstituted in this manner, the difference in NR CE-SDS % Main peak between the two conditions was ~45%, identical to the small-scale model results, which indicates that the reducing activity was preserved through this processing step. This is consistent with the experiment performed by Trexler-Schmidt et al. When purified mAb D (IgG2κ) was spiked into mAb A soluble cellular components, little reduction (0.9% reduction in % main peak) was observed, showing that mAb D reduction is more resistant to these conditions. Thus, the product will influence the degree of reduction observed in the harvest process. To test the effects of cell line and cell culture process independent of product, purified mAb A was spiked into mAb B and mAb C soluble cellular components. Although mAb A is susceptible to reduction in its own soluble cellular components, little reduction was observed when it was incubated in either mAb C or mAb B components (1.5% and 2.7% reduction in % main peak, respectively). All of the materials were carefully sparged with nitrogen during processing, and the soluble cellular components were prepared and used within 30 min of cell lysis. In addition, repeat analysis of the (mAb B) IgG2α spike into the mAb A (IgG1κ) yielded identical results, as did intermediate time points for the other conditions. The lack of reduction for these conditions must be due to differences in the reducing power of cellular component samples because the product is identical. Disulfide reducing ability of the cellular component sample could arise from differences in the cell line, such as differences in expression of thioredoxin or thioredoxin reductase, or differences in availability of NADPH due to regulation of the pentose phosphate pathway (PPP). Differences in reducing power of the soluble cellular component sample could also arise from the cell culture process differences, either indirectly, by influencing expression of thioredoxin, expression of thioredoxin reductase, and utilization of the PPP, or directly, by differing levels of redox active media components such as cystine/cysteine and copper. In the case of mAb C, the measured thioredoxin reductase activity is lower than that for mAb A (Table 1); however, this could be due to the lower cell density. In contrast, the cell density is similar for mAb B, and therefore the cell line and process can be determined to have a significant effect on reducing power. The presence of substantial thioredoxin reductase activity in these cell lysates is not unexpected because some apoptosis, which will release intracellular contents, inevitably occurs during cell culture. This means, however, that the difference in reducing power cannot be attributed to thioredoxin system activity alone because the thioredoxin reductase activity was higher in the mAb B lysate than the mAb A lysate. Therefore, either the other redox active components of the system have a major affect or substantial differences in the availability of NADPH exist. Taken together, the results suggest that the cell line (clone) or cell culture process play a key role in harvest-related reduction.

**Product properties.** As described above, the reconstituted extract model, demonstrates that striking differences exist in susceptibility to reduction among antibody products. Previously published studies have shown that antibody sub-classes differ in sensitivity to disulfide bond reduction. Differences in reduction susceptibility due to light chain type have not previously been observed for thioredoxin catalyzed reduction, but have been shown using chemical reductants. A chemical model system was developed to investigate antibody type (IgG1 and IgG2) and light chain type sensitivity to thioredoxin catalyzed reduction. As illustrated in Figure 4, reduction sensitivity is dependent on both antibody class and light chain type. Reduction sensitivity, in decreasing order, is IgG1λ, IgG1κ, IgG2α, IgG2κ. This trend held true for all of the additional molecules we have tested, and for different stoichiometric ratios of the reagents and antibody (data not shown). Sensitivity to antibody subclass has also been reported for other reductants, such as DTT. The reduction sensitivity trend

![Figure 3. Reduction Behavior of Different Products and Cell Lines over time. mAb A (triangles), mAb B (circles), and mAb C (squares).](image-url)
This phenomenon is caused by shearing of cells, resulting in the release of intracellular components, and requires an anaerobic environment. Different cell lines and processes have been demonstrated to have strikingly different reduction responses, e.g., mAb B soluble cellular components having less than 1/10th the reducing power of those of mAb A. The difference in reducing power cannot be attributed to differences in thioredoxin and thioredoxin reductase levels, as measurement of thioredoxin reductase shows that it is higher in some of the cell lysates that show no reduction. Therefore, these differences must stem from other redox active components in the media, or more likely, from differences in NADPH availability and regulation of the PPP.

The susceptibility of products to reduction by thioredoxin has been demonstrated to be dependent on antibody class and light chain type, IgG1λ > IgG1κ > IgG2λ > IgG2κ, with potentially some sequence dependency within each range. The susceptibility of the antibody classes to thioredoxin catalyzed reduction follows the same trend as antibody disulfide reduction by DTT. Therefore, a general understanding of product reducibility is available prior to expression of the product, and a more refined understanding of its susceptibility to reduction is possible with only micrograms of material in a chemically-defined system.

With the understanding of the reducing power of the cell line and process, screening of cell lines and cell culture conditions is possible. Combining process knowledge with the antibody class, a good understanding of the overall reduction behavior can be obtained early in process development.

### Materials and Methods

#### Materials

Cell culture fluid and purified antibodies were produced at Amgen using standard manufacturing procedures. Reagents were obtained from Sigma-Aldrich unless otherwise specified.

#### Cell shearing

Complete cell lysis of end of production (EOP) cell culture fluid, which contains both cells and the media containing product, was achieved by high-pressure homogenization using a Microfluidics M-110Y high shear fluid processor. Homogenization was performed with a single pass at 8,000–10,000 psi. Complete lysis was verified using the Roche Innovatis Cedex AS20 cell counter.

#### Small-scale reduction model

A 3 L glass stirred-tank bioreactor (Applikon Corporation) controlled by a customized DeltaV distributed control system (DCS) was used to evaluate harvest conditions. Processed cells were transferred to this bioreactor. Agitation was set at 250 rpm. Temperature was controlled to 8–10°C by passing chilled water through a thermal well in the bioreactor. Room temperature conditions were similar between the thioredoxin system and with DTT (Fig. 4 and 5). This comparison indicates that differences in reduction during harvest are the result of overall reducing potential of the system and the antibody type, and not any specific interactions between thioredoxin and certain antibody types.

Equipped with the knowledge of the reducibility trend IgG1λ > IgG1κ > IgG2λ > IgG2κ, a general understanding of the product contribution to the risk of process-induced reduction can be made prior to expression of products. This general understanding can be further refined by putting a small amount of purified product into either the chemically-defined thioredoxin reducing system, or by making kinetic measurements of the reducibility of the product by DTT.

### Discussion

Process-induced partial antibody disulfide reduction is an active topic of discussion in the literature and with regulatory authorities. This phenomenon is caused by shearing of cells, resulting in the release of intracellular components, and requires an anaerobic environment. Different cell lines and processes have been demonstrated to have strikingly different reduction responses, e.g., mAb B soluble cellular components having less than 1/10th the reducing power of those of mAb A. The difference in reducing power cannot be attributed to differences in thioredoxin and thioredoxin reductase levels, as measurement of thioredoxin reductase shows that it is higher in some of the cell lysates that show no reduction. Therefore, these differences must stem from other redox active components in the media, or more likely, from differences in NADPH availability and regulation of the PPP.

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### Table 1. End of production cell densities, viability and titer for mAbs A, B and C

| Product     | Viable Cell Density (cells/mL) | Viability (%) | Titer (g/mL) | Thioredoxin Reductase Activity (µmol/min/mL) |
|-------------|--------------------------------|---------------|--------------|-----------------------------------------------|
| IgG2κ (mAb C) | 7.0x10⁶                        | 75.4          | 1.3          | Below detection limit                          |
| IgG2λ (mAb B) | 17.2x10⁶                       | 62.4          | 4.7          | 0.16                                          |
| IgG1κ (mAb A) | 27.0x10⁶                       | 78.9          | 4.4          | 0.04                                          |

### Table 2. Influence of product and cell line/process on reduction

| Soluble Cellular Component | Purified mAb | Difference in NR CE-SDS % Main peak |
|----------------------------|--------------|------------------------------------|
| IgG1κ (mAb A)              | IgG1κ (mAb A) | 46.3%                             |
| IgG1κ (mAb A)              | IgG2κ (mAb D) | 0.9%                              |
| IgG2κ (mAb C)              | IgG1κ (mAb A) | 1.5%                              |
| IgG2λ (mAb B)              | IgG1κ (mAb A) | 2.7%                              |

Difference in NR CE-SDS % Main peak, 8 h, relative to initial.

### Figure 4. Influence of Product on Reduction using Thioredoxin System

Intact antibody, as measured by % Main peak in the NR CE-SDS analysis as a function of time.
During homogenization, a nitrogen (N₂) gas overlay was applied. One minute of homogenization to ensure complete cell breakage. CCF was transferred to a 250 mL polycarbonate bottle (Nalgene) for one minute of homogenization to remove existing monoclonal antibodies (mAbs). MabSelect SuRe™ Protein A affinity resin (GE Healthcare) was washed twice with an equilibration buffer of 100 mM NaCl, 25 mM Tris, pH 7.4, dried by vacuum over a nylon membrane, and applied in excess directly to the bottle. The mixture was placed on a rocker for 10 min to facilitate binding. The solution was centrifuged for 5 min x 1000 rpm in 50 mL conical tubes to pellet the resin. The supernatant was extracted from each tube and sparged with N₂ to form the soluble cellular components, and used within 30 min of production.

Sample antibody drug substance was added to a separate 15 mL polypropylene centrifuge tube and brought to a total volume of 7 mL with the soluble cellular component material to give a final antibody concentration of 3 mg/mL. An N₂ overlay was applied to each tube. The tubes were covered with laboratory paraffin film and placed in a digitally controlled water bath set at 10°C. One mL aliquots were pulled at 0, 4 and 8 h and immediately frozen at −80°C prior to analysis by NR CE-SDS.

**Reductase activity.** Thioredoxin reductase activity of lysates was assessed using a colorimetric 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) kit (Cayman Chemical). Briefly, lysed CCF was added to a pH 7 sample buffer containing 50 mM potassium phosphate, 50 mM potassium chloride, 1 mM EDTA, and 0.2 mg/mL bovine serum albumin, at a final dilution factor of 1:10. For each sample a matrix control was made by adding 20 μM sodium aurothiomalate (final concentration). Excess NADPH and 0.5 mM DTNB (final concentration) were added to each sample, matrix control, blank, and positive control (rat liver thioredoxin reductase). Light absorbance was monitored at 405 nm for 5 min, and the activity of thioredoxin reductase in μmol/min/mL was calculated by taking the difference in slopes between the sample and the matrix control, dividing by the extinction coefficient and path length, and multiplying by the dilution factor.

**Non-reduced CE-SDS.** Harvested cell culture fluid samples were prepared using an automated robotic platform, as previously described. Briefly, samples were centrifuged at 13,000 rpm for 1 min and loaded onto a Micro-Extractor Automated Instrument (MEA, PhyNexus). PhyTip® 200 μL Columns with 20 μL protein A affinity resin protein A tips were used to remove host cell proteins. Non-reducing sample buffer with a final concentration of 7 mM NEM, 57 mM sodium phosphate, 1.9% SDS, pH 6.5 was added to the purified samples. Incubation was set for 5 min at 60°C and samples were loaded onto a 30 cm bare fused silica capillary with a 20 cm effective length and 50 μm inner diameter using electrophoretic injection. Separation was performed using CE-SDS gel (Beckman Coulter) and 15 kV effective voltage, and detection was by UV light absorbance at 220 nm.

**Reconstituted extract model.** Cell culture fluid (CCF) depleted in antibody product was generated using a batch binding process to remove existing monoclonal antibodies (mAbs). CCF was transferred to a 250 mL polycarbonate bottle (Nalgene) and homogenized using a Tissue Tearor™ (Biospec Products) for one minute of homogenization to ensure complete cell breakage. Homogenization, a nitrogen (N₂) gas overlay was applied. MabSelect SuRe™ Protein A affinity resin (GE Healthcare) was washed twice with an equilibration buffer of 100 mM NaCl, 25 mM Tris, pH 7.4, dried by vacuum over a nylon membrane, and applied in excess directly to the bottle. The mixture was placed on a rocker for 10 min to facilitate binding. The solution was centrifuged for 5 min x 1000 rpm in 50 mL conical tubes to pellet the resin. The supernatant was extracted from each tube and sparged with N₂ to form the soluble cellular components, and used within 30 min of production.

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**Reduction by thioredoxin system.** The roles of thioredoxin and thioredoxin reductase (TR) have previously been described as nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular protein disulfide reductases. An in vitro lab-scale model using this complex was optimized using recombinant human thioredoxin (Sigma), NADPH (Calbiochem), in excess and thioredoxin reductase from rat liver (Sigma). A polypropylene 2 mL cryogenic vial (Corning) was sparged for 1 min with N₂ prior to being sealed in a borosilicate septa vial (I-Chem). In a separate 1.5 mL microcentrifuge tube, 825 μL TR solution (7 mM TRIS-HCl, pH 7.5 (Teknova) with 2 mM DTT (Geno Technology) at ambient temperature. Aliquots were taken at multiple time points and the reduction was quenched by immediately adding NEM (MP biomedical) to a final concentration of 25 mM. A non-reducing Caliper CE-SDS assay was performed to measure the level of reduction.

**Thioredoxin reductase activity.** Thioredoxin reductase activity of lysates was assessed using a colorimetric 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) kit (Cayman Chemical). Briefly, lysed CCF was added to a pH 7 sample buffer containing 50 mM potassium phosphate, 50 mM potassium chloride, 1 mM EDTA, and 0.2 mg/mL bovine serum albumin, at a final dilution factor of 1:10. For each sample a matrix control was made by adding 20 μM sodium aurothiomalate (final concentration). Excess NADPH and 0.5 mM DTNB (final concentration) were added to each sample, matrix control, blank, and positive control (rat liver thioredoxin reductase). Light absorbance was monitored at 405 nm for 5 min, and the activity of thioredoxin reductase in μmol/min/mL was calculated by taking the difference in slopes between the sample and the matrix control, dividing by the extinction coefficient and path length, and multiplying by the dilution factor.

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Non-reduced Caliper CE-SDS. A LabChip 90 (Caliper Life Sciences) was used to separate SDS bound proteins through a sieving polymer based on the hydrodynamic size of the SDS-protein complex.\(^7\) HT Protein Express Sample Buffer (Caliper Life Sciences) was combined with iodoacetamide (IAM) to a final IAM concentration of approximately 5 mM. A total of 5 \(\mu\)L antibody sample at approximately 1 mg/mL was mixed with 100 \(\mu\)L of the IAM containing sample buffer. The samples were incubated at 75°C for 10 min. The denatured proteins were analyzed by LabChip 90 with the “HT Protein Express 200” program.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Maggon K. Monoclonal antibody "gold rush". Curr Med Chem 2007; 14:1978-87; PMID:17691940; http://dx.doi.org/10.2174/092986707781368504
2. Reichert JM, Rosenzweig CJ, Faden LB, Dewitz MC. Monoclonal antibody successes in the clinic. Nat Biotechnol 2005; 23:1073-8; PMID:16151394; http://dx.doi.org/10.1038/nbt0905-1073
3. Kao YH, Hewitt DP, Tresler-Schmidt M, Laird MW. Mechanism of antibody reduction in cell culture production processes. Biotechnol Bioeng 2010; 107:622-32; PMID:20589844; http://dx.doi.org/10.1002/bit.22848
4. Koterba KL, Borgschulte T, Laird MW. Thioredoxin 1 is responsible for antibody disulfide reduction in CHO cell culture. J Biotechnol 2012; 157:261-7; PMID:22138638; http://dx.doi.org/10.1016/j.jbiotec.2011.11.009
5. Tresler-Schmidt M, Sargs S, Chiu J, Sz-Khoo S, Mun M, Kao YH, et al. Identification and prevention of antibody disulfide bond reduction during cell culture manufacturing. Biotechnol Bioeng 2010; 106:452-61; PMID:20718122
6. Mamathambika BS, Bardwell JC. Disulfide-linked protein folding pathways. Annu Rev Cell Dev Biol 2008; 24:211-35; PMID:18588487; http://dx.doi.org/10.1146/annurev.cellbio.24.110707.175333
7. Hunt G, Nashabeh W. Capillary electrophoresis sodium dodecyl sulfate nongel sieving analysis of a therapeutic recombinant monoclonal antibody: a biotechnology perspective. Anal Chem 1999; 71:2390-7; PMID:10405607; http://dx.doi.org/10.1021/ac981209m
8. Magnusson CG, Bjornstedt M, Holmgren A. Human IgG is substrate for the thioredoxin system: differential cleavage pattern of interchain disulfide bridges in IgG subclasses. Mole Immunol 1997; 34:709-17
9. Liu H, Chumsae C, Gara-Bulesco G, Hurkmans K, Radziejewski CH. Ranking the susceptibility of disulfide bonds in human IgG1 antibodies by reduction, differential alklylation, and LC-MS analysis. Anal Chem 2010; 82:5219-26; PMID:20491447; http://dx.doi.org/10.1021/ac100575n
10. Liu H, Zhong S, Chumsae C, Radziejewski C, Hsieh CM. Effect of the light chain C-terminal serine residue on disulfide bond susceptibility of human immunoglobulin G1A. Anal Biochem 2011; 408:277-83; PMID:20869344; http://dx.doi.org/10.1016/j.ab.2010.09.025
11. Montafar RE, Morrison SL. Influence of the isotype of the light chain on the properties of IgG: J Immunol 2002; 168:224-31; PMID:11751966
12. Chusainow J, Yang YS, Yeo JH, Toh PC, Asvadi Z, Wong NS, et al. A study of monoclonal antibody-producing CHO cell lines: what makes a stable high producer? Biotechnol Bioeng 2009; 102:1182-96; PMID:18979540; http://dx.doi.org/10.1002/bit.22158
13. Warm FM. Production of recombinant protein therapeutics in cultivated mammalian cells. Nat Biotechnol 2004; 22:1393-8; PMID:15529164; http://dx.doi.org/10.1038/nbt1026
14. Holmgren A. Thioredoxin. Annu Rev Biochem 1985; 54:237-71; PMID:3896121; http://dx.doi.org/10.1146/annurev.bi.54.070185.001321
15. Holmgren A. Thioredoxin and glutaredoxin systems. J Biol Chem 1989; 264:13963-6; PMID:2668278
16. Le ME, Vizel A, Hutterer KM. Automated sample preparation for CE-SDS. Electrophoresis 2013; (accepted); PMID:23423814; http://dx.doi.org/10.1002/elps.201200644
17. Chen X, Tang K, Lee M, Flynn GC. Microchip assays for screening monoclonal antibody product quality. Electrophoresis 2008; 29:4993-5002; PMID:19130579; http://dx.doi.org/10.1002/elps.200800324