about 100 mM (e.g., 10 mM, 20 mM, 50 mM, etc.), and physiological salt concentrations (e.g., about 0.15 mM NaCl), and at pH from 5.0-9.0.

In a preferred embodiment of the invention, an intermediate wash step is performed. It has been discovered that reduced levels of HCP in general, and in particular, procathepsin L, can be achieved by using an intermediate wash buffer comprising, in part, the same buffer as the CEX elution buffer. The improved reduction of HCP in general, and in particular procathepsin L, results in part, from the increased conductivity in the intermediate wash buffer. Increasing the conductivity of the intermediate wash buffer causes charge displacement of the HCP(s), which has a lower pI relative to ~~the~~ that of the antibody, thus causing the weaker binding HCP(s) to wash through the column. Increased clearance of the weaker binding impuities, *e.g.*, HCP(s) including procathepsin L, in turn provides more binding area for the target substance, *e.g.*, the antibody. In other embodiments, the intermediate wash buffer contains from about 40% to about 50% elution buffer and from about 50% to about 60% water for injection. In further embodiments, the intermediate wash buffer contains 45% elution buffer and 55% water for injection. In one embodiment, the wash buffer used in the intermediate wash is 20 mM $Na_2PO_4$, 150 mM sodium chloride, pH 7.

Following a plurality of washes, the antibody is eluted from the first cationic exchange material such that a first eluate having a reduced level of HCP is obtained. The first eluate also has a reduced level of procathepsin L in view of the intermediate wash step. In one embodiment, the first eluate obtained using the method of the invention comprises an about 3 to an about 5 fold decrease in HCP levels in comparison to a comparable step of process A. In another embodiment, the first eluate obtained using the method of the invention comprises an about 2 to an about 3 fold decrease in cathepsin L activity in comparison to a comparable step of process A. In one embodiment, the first eluate comprises a range of about 90 to about 100 fold less HCP than the mixture as determined by a HCP ELISA. In another embodiment, the first eluate comprises cathepsin L activity ranging from between about 25 to about 60 RFU/s/mg of antibody as measured by a cathepsin L kinetic assay

In a preferred embodiment, the initial eluate comprising the antibody is passed over a second IE material and a flowthrough comprising a further reduced level of HCP is obtained. In some embodiments, the second IE step may be batch purification as described *infra*. In other embodiments, the second IE step comprises loading the first eluate onto a second ion exchange chromatography column, washing the column and obtaining a first flowthrough. The second IE material may be anion exchange (AEX) resin, *e.g.*, Q sepharose column. In some embodiments, between about 30 g antibody per L resin and about 40 g antibody per L or resin is loaded onto the anion exchange column. Increasing the loading amount between about 40 g antibody per L resin and

and

to be between 0.0144 ~~to~~ 1.04 RFU/sec. This activity was correlated to the amount of adalimumab present in the test sample; hence results are report as RFU/sec/mg adalimumab. Optimum activation conditions to achieve the maximum fluorescent signal were developed for each process intermediate sample using JMP software derived DOE experiments. The recommended activation conditions for this assay are summarized in Table ~~16.~~ 18.

Materials and methods

*Preparation of 500 mM DTT stock solution*

7.7 grams of Ultrapure DTT (Invitrogen) was added into 90 mL of Milli-Q water and mixed until homogenous. The solution was topped up the solution with Milli-Q Water to a final volume of 100 mL. This 500 mM DTT stock was then aliquoted and stored at –80°C.

*Preparation of the activation buffer (25 mM NaOAc, 5 mM DTT, 1 mM EDTA pH 5.5)*

3.44 grams of sodium acetate (J.T. Baker), 0.38 grams of EDTA (J.T. Baker) and 950 mL Milli-Q water were added to a proper container and mixed until completely homogonous. The pH of the buffer was adjusted to 5.5 with 1 M HCl, and brought up to the final volume of 1 L in a volumetric flask. The buffer was filtered through a 0.22 μm filter and stored at 4°C prior to use. 500 μL of DTT stock solution (500 mM described above) was added to 50 mL of buffer to a final concentration of 5 mM at the day of use.

*Preparation of dextran sulfate + 0.1% sodium azide stock solution*

1 gram of dextran sulfate (EM Science) was added into 90 mL of Milli-Q water and mixed by until homogenous. 100 μL sodium azide was added from a 1 mg/mL stock solution (J.T. Baker). The solution was topped up to a final volume of 100 mL. This solution was then aliquoted and stored at –80°C.

*Kinetic assay set-up*

Samples to be tested for cathepsin L activity require activation of the proenzyme (procathepsin L) to active enzyme (cathepsin L). This was accomplished by diluting samples in activation buffer, adding dextran sulfate and incubating at 37°C for an appropriate time (details discussed in below). After activation, samples can be stored at –80°C and remain stable. Optimal activation conditions determined for in-process samples are shown in Table 18.

Table 18: Summary of refined activation conditions for in-process samples

end of the 30 minutes measurement time, resulting in a lower $R^2$ value of 0.91. Therefore, careful sample dilution is critical to maintain linear hydrolysis rates.

Assays were also performed to confirm that the kinetic assay using cathepsin activity to determine the level of procathepsin ~~A~~ L were compliant with ICH guidelines, including precision analysis, including repeatbility precision. Furthermore, it was deteremined that the type of container, *e.g.*, glass and polypropylene vials influences of cathepsin L activity. The results suggest that higher levels of cathepsin L are achieved when incubating in a polypropylene container as opposed to a glass container. In both cases, the addition of 0.5 μg/mL dextran sulfate was required for procathepsin L activation at pH 5.5.

In sum, the precision of the kinetic assay demonstrates that this assay is valid for detection of potential cathepsin L activity of adalimumab process intermediates.

This application is related to U.S. Patent Nos. 6,090,382, 6,258,562, and 6,509,015. This application is also related to U.S. Patent Application Serial No. 09/801,185, filed March 7, 2001; U.S. Patent Application Serial No. 10/302,356, filed November 22, 2002; U.S. Patent Application Serial No. 10/163657, filed June 5, 2002; and U.S. Patent Application Serial No. 10/133715, filed April 26, 2002; U.S. Patent Application Serial No. 10/222140, filed August 16, 2002; U.S. Patent Application Serial No. 10/693233, filed October 24, 2003; U.S. Patent Application Serial No. 10/622932, filed July 18, 2003; U.S. Patent Application Serial No. 10/623039, filed July 18, 2003; U.S. Patent Application Serial No. 10/623076, filed July 18, 2003; U.S. Patent Application Serial No. 10/623065, filed July 18, 2003; U.S. Patent Application Serial No. 10/622928, filed July 18, 2003; U.S. Patent Application Serial No. 10/623075, filed July 18, 2003; U.S. Patent Application Serial No. 10/623035, filed July 18, 2003; U.S. Patent Application Serial No. 10/622683, filed July 18, 2003; U.S. Patent Application Serial No. 10/622205, filed July 18, 2003; U.S. Patent Application Serial No. 10/622210, filed July 18, 2003; and U.S. Patent Application Serial No. 10/623318, filed July 18, 2003. This application is also related to PCT/US05/12007, filed April 11, 2005. The entire contents of each of these patents and patent applications are hereby incorporated herein by reference.

**EQUIVALENTS**

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.