least four members; b) spiking each member of the dilution series with a constant amount of a template DNA distinguishable from host cell DNA; c) performing a PCR assay on each member of the dilution series and on the constant amount of the template DNA in the absence of any sample; d) generating a polyanionic inhibitor standard curve; e) comparing the PCR assay results of the dilution series to the PCR assay results of the constant amount of the template DNA in the absence of any sample; and f) identifying the concentration of polyanionic PCR inhibitor in the sample. In some examples, the concentration of polyanionic PCR inhibitor in the sample is a range defined by the concentration of polyanionic PCR inhibitor in the least diluted member of the dilution series showing complete spike recovery and the most diluted member of the dilution series not showing complete spike recovery. In some examples, the number of members in the dilution series is 5, 6, 7, 8, 9, 10, 12, 15, or 20, thereby narrowing the range of concentration of the polyanionic PCR inhibitor in the sample relative to the range provided by assaying fewer members of a dilution series. In some examples, the constant amount of a template DNA is at least 100 pg. In some examples, the polyanionic PCR inhibitor is a sulfone (sulfonate) compound, such as polyvinyl sulfonate. In some examples, polyvinyl sulfonate is the polyanionic PCR inhibitor used in generating the polyanionic inhibitor standard curve and the concentration of polyanionic PCR inhibitor in the sample is in units of polyvinyl sulfonate concentration equivalents. Some examples further comprise spiking a sample diluted sufficiently to recover amplification with one or more amounts of a polyanionic PCR inhibitor such as polyvinyl sulfonate to demonstrate that with added inhibitor, the recovery of amplification is impaired or lost.

[0009] Another example of the disclosure is drawn to a method for removing a polyanionic PCR inhibitor (a polyanionic impurity) from a buffer solution comprising: a) preparing a buffer solution of an acidic buffering species, a basic buffering species, or a combination thereof; b) contacting the buffer solution with an anion exchange medium; and c) separating the buffer solution from the polyanionic impurity, thereby removing the polyanionic impurity from the buffer solution. A related example of the disclosure provides a method for removing a polyanionic PCR inhibitor from a buffer solution comprising: a) preparing a buffer solution of an acidic buffering species, a basic buffering species, or a combination thereof; b) contacting the buffer solution with a mixed mode resin; and c) separating the buffer solution from the polyanionic impurity, thereby removing the polyanionic impurity from the buffer solution. In either of the foregoing methods for removing a polyanionic PCR inhibitor, the volume of buffer subjected to the method is not limiting and may extend from small analytical volumes in the range of milliliters to commercial scale buffer preparations involving many liters. In some examples of either removal method, the polyanionic impurity is a sulfone (sulfonate) compound, such as a polyvinyl

sulfonate. In some examples of either removal method, the buffer solution is a Good's buffer solution, such as a 2-(N-morpholino)-ethanesulfonic acid (MES) buffer solution. In some examples of either removal method, the buffer solution comprises a buffering salt or acid species of the buffering salt. Some examples of each of the removal methods further comprise adding at least one modifying compound to the buffer solution. In some examples of either removal method, the modifying compound is a non-buffering salt, an excipient, or both.

[0010] In some examples of the removal method involving an anion exchange medium, the anion exchange medium is diethylaminoethyl-modified matrix, Dimethylaminoethyl-modified matrix, dimethylaminopropyl-modified matrix, polyethyleneimine-modified matrix, quaternized polyethyleneimine-modified matrix, fully quaternized amine-modified matrix, anion exchange modified diatomaceous earth-containing depth filters, anion exchange membrane adsorbers, salt tolerant anion exchange membrane adsorbers, Macro-Prep 25Q, TSK-Gel Q, Poros Q, Q Sepharose Fast Flow, Q HyperD, Q Zirconia, Source 30Q, Fractogel EMD TMAE, Express-Ion Q, DEAE Sepharose Fast Flow, Poros 50 D, Fractogel EMD DEAE (M), MacroPrep DEAE Support, DEAE Ceramic HyperD 20, Toyopearl DEAE 650 M, Capto Q, Sartobind Q membrane absorber, Posidyne charged membrane, Amberlite® (polyamine)-modified matrix, anion exchange-modified diatomaceous earth containing depth filters, anion exchange membrane adsorbers, salt tolerant anion exchange membrane adsorbers, Macro-Prep 25Q, TSK-Gel Q, Poros Q, Q Sepharose Fast Flow, Q HyperD, Q Zirconia, Source 30Q, Fractogel EMD TMAE, Express-Ion Q, DEAE Sepharose Fast Flow, Poros 50 D, Fractogel EMD DEAE (M), MacroPrep DEAE Support, DEAE Ceramic HyperD 20, Toyopearl DEAE 650 M, Capto Q, Sartobind Q membrane absorber, Posidyne charged membrane, Amberlite® (iminodiacetic acid)-modified matrix, Amberlite® Type I (trialkylbenzyl ammonium)-modified matrix, Amberlite® Type II (dimethyl-2-hydroxyethylbenzyl ammonium)-modified matrix, Dowex® (polyamine)-modified matrix, Dowex® Type I (trimethylbenzyl ammonium)-modified matrix, Dowex® Type II (dimethyl-2-hydroxyethylbenzyl ammonium)-modified matrix, Dowex® (mixed bed),Capto™ Adhere anion exchange multimodal medium, Capto® Adhere Anion Exchange Multi Mode, PPA Hypercel, HEA Hypercel, or Duolite® (polyamine)-modified matrix. In some examples of the removal method involving mixed mode resin, the mixed mode resin is Capto® Adhere Anion Exchange Multi Mode resin, PPA Hypercel resin, or HEA Hypercel resin. Any matrix known in the art is suitable for use in the removal methods, including but not limited to, cellulose, agarose, Sepharose®, methacrylic polymer, ceramic scaffolds with polymerized hydrogels, and proprietary matrices.

[0011] Some examples of the removal method involving an anion exchange medium provide an anion exchange medium that binds up to 15 mg, 9 mg, or 3 mg PVS per mL of anion exchange medium. Some examples of the removal method involving a mixed mode resin provide a mixed mode resin that binds up to 15 mg, 9 mg, or 3 mg PVS per mL of mixed mode resin. In some examples of the removal method involving an anion exchange medium, the anion exchange medium is a polycationic compound is a titrant that forms a complex with the polyanionic impurity (analyte). In some examples, the anion exchange medium is a quaternary ammonium-based polymer. In some examples of the removal method involving an anion exchange medium, the polycationic compound is added in an amount sufficient to at least reach the equivalence point in titrating the polyanionic impurity analyte. As used herein, the equivalence point is the point in a titration when sufficient titrant is added to bind all of the analyte and is a synonym for titration point.

[0012] Yet another example of the disclosure is drawn to a method for removing a polyanionic buffer impurity from a protein solution comprising: a) adjusting the pH of a protein solution containing an anionic buffer impurity to a pH less than the isoelectric point of the protein by no more than 4 pH units; b) contacting the protein solution with an anion exchange medium; and c) separating the protein from the anionic buffer impurity. In a related example, the disclosure provides a method for removing a polyanionic buffer impurity from a protein solution comprising: a) adjusting the pH of a protein solution containing an anionic buffer impurity to a pH less than the isoelectric point of the protein by no more than 4 pH units; b) contacting the protein solution with a mixed mode resin; and c) separating the protein from the anionic buffer impurity. In some examples of either of these pH-based removal methods, the pH is adjusted to be lower than the isoelectric point of the protein by no more than 2 pH units. In some examples of the pH-based removal method involving an anion exchange medium, the anion exchange medium is diethylaminoethyl-modified matrix, Dimethylaminoethyl-modified matrix, dimethylaminopropyl-modified matrix, polyethyleneimine-modified matrix, quaternized polyethyleneimine-modified matrix, fully quaternized amine-modified matrix, an anion exchange-modified diatomaceous earth-containing depth filter, an anion exchange membrane adsorber, a salt-tolerant anion exchange membrane adsorber, Macro-Prep 25Q, TSK-Gel Q, Poros Q, Q Sepharose Fast Flow, Q HyperD, Q Zirconia, Source 30Q, Fractogel EMD TMAE, Express-Ion Q, DEAE Sepharose Fast Flow, Poros 50 D, Fractogel EMD DEAE (M), MacroPrep DEAE Support, DEAE Ceramic HyperD 20, Toyopearl DEAE 650 M, Capto Q, Sartobind Q membrane absorber, Posidyne charged membrane, Amberlite® (polyamine)-modified matrix, Amberlite® (iminodiacetic acid)-modified matrix, Amberlite® Type I (trialkylbenzyl ammonium)-modified matrix, Amberlite® Type II (dimethyl-2-hydroxyethylbenzyl ammonium)-modified matrix, Dowex®

(polyamine)-modified matrix, Dowex® Type I (trimethylbenzyl ammonium)-modified matrix, Dowex® Type II (dimethyl-2-hydroxyethylbenzyl ammonium)-modified matrix, Dowex® (mixed bed), Capto® Adhere Anion Exchange Multi Mode, PPA Hypercel, HEA Hypercel, or Duolite® (polyamine)-modified matrix. As noted above, any matrix known in the art is suitable for use in the methods according to the disclosure. In some examples of the pH-based removal method involving mixed mode resin, the mixed mode resin is Capto® Adhere Anion Exchange Multi Mode, PPA Hypercel, or HEA Hypercel.

[0013] Still another example of the disclosure is a titration method for detecting a polyanionic enzyme inhibitor in a buffer solution comprising: (a) contacting a buffer solution with a polycationic compound; (b) adding a polyanionic compound to the solution in (a), wherein the polyanionic compound exhibits a change in a detectable property when complexed to a polycationic compound compared to the uncomplexed polyanionic compound; (c) repeating steps (a) and (b) with varying concentrations of the buffer solution or varying concentrations of the polycationic compound; and (d) detecting the change in the detectable property at the titration point, thereby detecting the polyanionic enzyme inhibitor. In some examples, the buffer concentration is varied, thereby creating a dilution series of the buffer. In some examples, the concentration of the polycationic compound is varied. In some examples, the polyanionic enzyme inhibitor is polyvinyl sulfonate or a derivative thereof. In some examples, the polycationic compound is a pH-independent polycationic compound or a pH-dependent polycationic compound. In some examples, the pH-independent polycationic compound is a quaternary ammonium-based polymer. In some examples, the pH-dependent polycationic compound is a polyamine. In some examples, the quaternary ammonium-based polymer is hexadimethrine bromide (HDBr), poly(diallyl)dimethylammonium chloride (pDADMAC), or methylglycol chitosan. In some examples, the quaternary ammonium-based polymer is hexadimethrine bromide (HDBr) or poly(diallyl)dimethylammonium chloride (pDADMAC). In some examples, the quaternary ammonium-based polymer is hexadimethrine bromide (HDBr).

[0014] In some examples, the polyanionic compound is a dye. In some examples, the dye is Eriochrome Black T (ECBT), Eriochrome Blue Black R (Calcon) or Sulfonazo sodium salt. In some examples, the dye is Eriochrome Black T (ECBT). In some examples, the buffer is a Good's buffer. In some examples, the Good's buffer comprises a polyethane sulfonic acid derivative or a polypropane sulfonic acid derivative. In some examples, the Good's buffer is MES, Bis-tris methane, ADA, Bis-tris propane, PIPES, ACES, MOPSO, Cholamine chloride, MOPS, BES, AMPB, HEPES, DIPSO, MOBS, Acetamidoglycine, TAPSO,

TEA, POPSO, HEPPSO, EPS, HEPPS, Tricine, Tris, Glycinamide, Glycylglycine, HEPBS, Bicine, TAPS, AMPB, CHES, CAPSO, AMP, CAPS, or CABS. In some examples, the uncomplexed polyanionic compound is detected using fluorometry or spectrophotometry. In some examples, the method further comprises determining the concentration of the polyanionic enzyme inhibitor from the quantity of polyanionic compound required to detect the change in the detectable property.

[0015] Another example of the disclosure is drawn to a method for quantification of a polyanionic PCR inhibitor in a sample comprising: (a) contacting a sample comprising a polyanionic PCR inhibitor with at least one aliquot of a polycationic compound; (b) adding a polyanionic indicator dye in an amount sufficient to detect the free form of the dye; and (c) quantifying the polyanionic PCR inhibitor based on the amount of polycationic compound needed to detect the free form of the polyanionic indicator dye. A related example of the disclosure is directed to a method of removing a polyanionic impurity in a sample comprising: (a) contacting a fluid comprising a polyanionic impurity with a polycationic counterion; and (b) separating the fluid from the polyanionic impurity complexed to the polycationic counterion, thereby removing the polyanionic impurity from the fluid. In some examples, the polyanionic impurity is a polyanionic PCR inhibitor. In some examples, the complex of polyanionic impurity and polycationic counterion is removed by precipitation. In some examples, the polycationic counterion is derivatized by attachment to a member of a binding pair or a magnetic particle to facilitate removal of the complex of polyanionic impurity and polycationic counterion from the fluid. Exemplary binding pairs include, but are not limited to, antigen/antibody pairs, biotin/streptavidin, magnetic particle/iron-containing material, polyhistidine/metal ion (*e.g.*, nickel) pairs, and the like.

[0016] Other features and advantages of the disclosed subject matter will be apparent from the following detailed description and figures, and from the claims.

Brief Description of the Drawings

[0017] **Figure 1.** DNA assay spike recovery inhibition versus PVS standards.

[0018] **Figure 2.** Dilution series spike recovery analysis compared to standard curve to provide PVS concentrations.

[0019] **Figure 3.** Spike recovery data for MES fractions.

[0020] **Figure 4.** Spike recovery data for MES fractions.

[0021] **Figure 5.** Spike recovery data for MES fractions using AEX resin.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for quantification of a polyanionic PCR inhibitor in a sample comprising:
    a) Preparing a dilution series of a sample comprising at least four members;
    b) Spiking each member of the dilution series with a constant amount of a template DNA distinguishable from host cell DNA;
    c) Performing a PCR assay on each member of the dilution series and on the constant amount of the template DNA in the absence of any sample;
    d) Generating a polyanionic inhibitor standard curve;
    e) Comparing the PCR assay results of the dilution series to the PCR assay results of the constant amount of the template DNA in the absence of any sample; and
    f) Identifying the concentration of polyanionic PCR inhibitor in the sample.
2. The method of claim 1 wherein the concentration of polyanionic PCR inhibitor in the sample is a range defined by the concentration of polyanionic PCR inhibitor in the least diluted member of the dilution series showing complete spike recovery and the most diluted member of the dilution series not showing complete spike recovery.
3. The method of claim 2 wherein the number of members in the dilution series is 5, 6, 7, 8, 9, 10, 12, 15, or 20, thereby narrowing the range of concentration of the polyanionic PCR inhibitor in the sample relative to the range provided in claim 2.
4. The method of claim 1 wherein the constant amount of a template DNA is at least 100 pg.
5. The method of claim 1 wherein the polyanionic PCR inhibitor is a sulfonate compound.
6. The method of claim 5 wherein the sulfonate compound is polyvinyl sulfonate.
7. The method of claim 1 wherein polyvinyl sulfonate is the polyanionic PCR inhibitor used in generating the polyanionic inhibitor standard curve and the

concentration of polyanionic PCR inhibitor in the sample is in units of polyvinyl sulfonate concentration equivalents.