SUMMARY

[009] In one aspect, the present disclosure relates to a method for detecting carboxylesterase activity of contaminating host cell protein in a sample comprising a recombinant protein of interest produced in a eukaryotic cell comprising (a) providing at least one sample comprising a recombinant protein of interest produced in a eukaryotic cell in cell culture and at least one contaminating host cell protein; (b) contacting the at least one sample with a reaction solution (comprising a hydrophilic substrate) to form a reaction mixture, wherein the reaction solution comprises: (i) a buffer having a pH of about pH 4 to about pH 8, (ii) a hydrophilic substrate, wherein the substrate is a saturated unbranched-chain fatty acid (C6-C12) ester of 1-hydroxypyrene-3,6,8-trisulfonic acid or a salt thereof (substrate HPTS ester), and (iii) optionally a non-buffering salt; (c) incubating the sample and the substrate in the reaction mixture; (d) detecting carboxylesterase activity of the at least one contaminating host cell protein by measuring hydrolysis of the substrate HPTS ester and detecting the fluorescence intensity of the released chromophore 1-hydroxypyrene-3,6,8-trisulfonic acid or a salt thereof (HPTS); optionally measuring hydrolysis by detecting the fluorescence intensity of the released chromophore HPTS over time, while incubating the sample and the substrate in the reaction mixture according to step (c). In certain examples the sample and the substrate in the reaction mixture are incubated for any time period between 2 min and 5 hours, 2 min and 3 hours, 2 min and 2 hours, or 2 min and 0.5 hours; and/or the reaction mixture has a volume of 300 µl or less. According to the method, multiple reaction mixtures may be analysed in parallel, preferably in a volume of 300 µl or less.

[010] The fluorescence of the released chromophore HPTS is determined preferably using an excitation wavelength within a range of 401-405 nm and an emission wavelength within a range of 510-516 nm. In preferred examples, the substrate HPTS ester is selected from the group consisting of 1-octanoyloxy-pyrene-3,6,8-trisulfonic acid or a salt thereof (OPTS), 1-nonaoyloxy-pyrene-3,6,8-trisulfonic acid or a salt thereof and 1-decanoyloxy-pyrene-3,6,8-trisulfonic acid or a salt thereof. In a more preferred example, the substrate HPTS ester is selected from the group consisting of 1-octanoyloxy-pyrene-3,6,8-trisulfonic acid trisodium salt (OPTS), 1-nonaoyloxy-pyrene-3,6,8-trisulfonic acid trisodium salt and 1-decanoyloxy-pyrene-3,6,8-trisulfonic acid trisodium salt. Preferably the substrate HPTS ester is used at a final concentration in the reaction mixture of 50 µM or less or preferably 30 µM or less.

[011] In preferred examples, the method further comprises (bi) contacting the at least one sample comprising a recombinant protein of interest produced in a eukaryotic cell in cell culture and at least one contaminating host cell protein of step (a) in a separate reaction set-up with a reaction solution comprising a lipophilic substrate to form a reaction mixture, wherein the reaction solution comprises: (i) a buffer having a pH of about pH 4 to about pH 8, (ii) a lipophilic substrate comprising the chromophore 4-methylumbelliferyl (4-MU) in the form of a 4-MU ester, wherein the 4-MU ester is a saturated unbranched-chain fatty acid (C6-C16) 4-MU ester, (iii) optionally a non-buffering salt, and (iv) a non-denaturing detergent not having an ester-bond, wherein the detergent is a non-ionic or zwitterionic detergent, (ci) incubating the sample and the substrate in the reaction mixture of step (bi); and (di) detecting lipase activity of the at least one contaminating host cell protein by measuring hydrolysis of the substrate 4-MU ester and detecting the fluorescence intensity of the released

chromophore 4-MU; optionally measuring hydrolysis by detecting the fluorescence intensity of the released chromophore 4-MU over time, while incubating the sample and the substrate in the reaction mixture according to step (ci).

[012] The lipophilic substrate is preferably selected from the group consisting of 4-methylumbelliferyl octanoate, 4-methylumbelliferyl nonanoate, 4-methylumbelliferyl decanoate (4-MUD), 4-methylumbelliferyl undecanoate and 4-methylumbelliferyl dodecanoate. Micelle formation improves lipase activity and solubilization of the lipophilic substrate. Thus, according to the disclosure the detergent used in the reaction solution comprising the lipophilic substrate has a final concentration in the reaction mixture above its critical micelle concentration in the reaction mixture. In certain examples, the detergent is selected from the group consisting of CHAPS, CHAPSO and Zwittergent, preferably CHAPS. In case the detergent is CHAPS, it is preferably provided at a final concentration in the reaction mixture of about 8 mM to about 20 mM, preferably at about 8 mM to about 15 mM, more preferably at about 10 mM. In certain examples the detergent is not polyethylene glycol tert-octylphenyl ether (Triton X-100) and not polyethylene glycol nonylphenyl ether (NP-40).

[013] The buffer used in the method of the disclosure (using the hydrophilic and/or lipophilic substrate) may comprises one or more buffer substances selected from the group consisting of a formic acid, acetic acid, lactic acid, citric acid, malic acid, maleic acid, glycine, glycylglycine, succinic acid, TES (2-{[tris(hydroxyme-thyl)methyl]amino}ethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid), PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)), MES (2-(N-morpholino)ethanesulfonic acid), Tris base, Tris, Bis-Tris, Bis-Tris-Propane, Bicine (N,N-bis(2-hydroxyethyl)glycine), HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid), TAPS (3-([tris(hydroxymethyl)methyl]amino}propanesulfonic acid), Tricine (N-tris(hydroxymethyl)methylglycine), Na_2HPO_4 and NaH_2PO_4. In certain examples, the buffer has a pH of about 5 to about 7.5, preferably the buffer has a pH of about 5.5 to about 7.0. The buffer may also be a multi-component buffer having a buffering range from at least about pH 5 to at least about pH 7.5, preferably from at least about pH 4 to at least about pH 8.

[014] The optionally non-buffering salt may be selected from the group consisting of NaCl, KCl and $CaCl_2$, preferably the non-buffering salt is NaCl or KCl. Moreover, the non-buffering salt may have a concentration of about 100 mM to about 200 mM, preferably about 130 mM to about 170 mM, more preferably about 140 mM to about 150 mM in the reaction mixture. In certain examples, the ionic strength of the non-buffering salt is about 200 mM or less in the reaction mixture, preferably about 150 mM or less in the reaction mixture; and/or the cumulative ionic strength of the buffer and the non-buffering salt in the reaction mixture is about 450 mM or less, preferably about 400 mM or less, more preferably about 350 mM or less in the reaction mixture.

[015] The at least one sample according to certain examples is a harvested cell culture fluid (HCCF), an in-process control (IPC) sample, a UF/DF filtrate, a drug substance sample or a drug product sample. In preferred examples, the recombinant protein of interest is produced in a CHO cell and the at least one contaminating host cell protein is a CHO host cell protein (CHOP). According to the disclosure the recombinant protein of interest is not a carboxylesterase or a lipase and/or an

enzyme having carboxylesterase or lipase activity. Preferably, the recombinant protein of interest is selected from the group consisting of an antibody, an antibody fragment, an antibody derived molecule and a fusion protein.

[016] In another aspect, the present disclosure further provides a method of manufacturing a recombinant protein of interest comprising the steps of (i) cultivating a eukaryotic cell expressing a recombinant protein of interest in cell culture; (ii) harvesting the recombinant protein of interest; (iii) purifying the recombinant protein of interest; and (iv) optionally formulating the recombinant protein of interest into a pharmaceutically acceptable formulation suitable for administration; and (v) obtaining at least one sample comprising the recombinant protein of interest in steps (ii), (iii) and/or (iv); wherein the method further comprises detecting carboxylesterase activity in a sample comprising the recombinant protein of interest and at least one contaminating host cell protein comprising: (a) providing the at least one sample obtained in step (v) comprising the recombinant protein of interest produced in a eukaryotic cell in cell culture and at least one contaminating host cell protein; (b) contacting the at least one sample with a reaction solution comprising a hydrophilic substrate to form a reaction mixture, wherein the reaction solution comprises: (i) a buffer having a pH of about pH 4 to about pH 8, (ii) a hydrophilic substrate, wherein the substrate is a saturated unbranched-chain fatty acid (C6-C12) ester of 1-hydroxypyrene-3,6,8-trisulfonic acid or a salt thereof (substrate HPTS ester), and (iv) optionally a non-buffering salt; (c) incubating the sample and the substrate in the reaction mixture; and (d) detecting carboxylesterase activity of the at least one contaminating host cell protein by measuring hydrolysis of the substrate HPTS ester and detecting the fluorescence intensity of the released chromophore HPTS; wherein the method optionally further comprises detecting lipase activity in a sample comprising (bi) contacting the at least one sample comprising a recombinant protein of interest produced in a eukaryotic cell and at least one contaminating host cell protein of step (a) in a separate reaction set-up with a reaction solution comprising a lipophilic substrate to form a reaction mixture, wherein the reaction solution comprises; (i) a buffer having a pH of about pH 4 to about pH 8, (ii) a lipophilic substrate comprising the chromophore 4-methylumbelliferyl (4-MU) in the form of a 4-MU ester, wherein the 4-MU ester is a saturated unbranched-chain fatty acid (C6-C16) 4-MU ester, (iii) optionally a non-buffering salt, and (iv) a non-denaturing detergent not having an ester-bond, wherein the detergent is a non-ionic or zwitterionic detergent, (ci) incubating the sample and the substrate in the reaction mixture; (di) detecting lipase activity of the at least one contaminating host cell protein by measuring hydrolysis of the 4-MU ester and detecting the fluorescence intensity of the released chromophore 4-MU; optionally measuring hydrolysis by detecting the fluorescence intensity of the released chromophore HPTS and/or 4-MU over time, while incubating the sample and the substrate in the reaction mixture according to step (c) or (ci), respectively.

[017] The method for manufacture according to the disclosure may further comprise obtaining at least one sample comprising the recombinant protein of interest in step (ii), wherein the sample is a harvested cell culture fluid (HCCF) or a cell lysate; in step (iii), wherein the sample is an in-process control (IPC) sample; and/or in step (iv), wherein the sample is a UF/DF sample, a drug substance sample or a drug product sample. In a preferred example, the method comprises obtaining at least one sample comprising the recombinant protein of interest produced in a eukaryotic cell in cell culture

and at least one contaminating host cell protein in step (iii), comprising obtaining at least one sample before and after affinity chromatography, before and after acid treatment, before and after depth filtration, and/or before and after ion exchange chromatography, preferably anion exchange chromatography or cation exchange chromatography.

[018] In yet another aspect, the disclosure relates to a kit for determining contaminating carboxylesterase and/or lipase activity in a sample comprising a recombinant protein of interest comprising: (i) a buffer having a pH of about pH 4 to about pH 8; and (ii) a hydrophilic substrate and a lipophilic substrate, wherein (a) the hydrophilic substrate is a saturated unbranched-chain fatty acid (C6-C12) ester of 1-hydroxypyrene-3,6,8-trisulfonic acid or a salt thereof (substrate HPTS ester); and (b) the lipophilic substrate comprises the chromophore 4-methylumbelliferyl (4-MU) in the form of a 4-MU ester, wherein the 4-MU ester is a saturated unbranched-chain fatty acid (C6-C16) 4-MU ester (substrate 4-MU ester); and optionally (iii) a non-buffering salt; and/or (iv) a non-denaturing detergent not having an ester-bond, wherein the detergent is a non-ionic or zwitter-ionic detergent.

[019] In certain examples the hydrophilic substrate HPTS ester in the kit is selected from the group consisting of 1-octanoyloxy-pyrene-3,6,8-trisulfonic acid or a salt thereof (OPTS), 1-nonaoyloxy-pyrene-3,6,8-trisulfonic acid or a salt thereof and 1-decanoyloxy-pyrene-3,6,8-trisulfonic acid or a salt thereof (such as 1-octanoyloxy-pyrene-3,6,8-trisulfonic acid trisodium salt (OPTS), 1-nonaoyloxy-pyrene-3,6,8-trisulfonic acid trisodium salt or 1-decanoyloxy-pyrene-3,6,8-trisulfonic acid trisodium salt); and/or the lipophilic substrate 4-MU ester is selected from the group consisting of 4-methylumbelliferyl octanoate, 4-methylumbelliferyl nonanoate, 4-methylumbelliferyl decanoate (4-MUD), 4-methylumbelliferyl undecanoate and 4-methylumbelliferyl dodecanoate. The kit according to the disclosure may further one or more microtiter plate having 96 wells or a multiple of 96 wells.

[020] In yet another aspect the disclosure relates to a use of a hydrophilic substrate HPTS ester or a hydrophilic substrate HPTS ester and a lipophilic substrate 4-MU ester as a substrate for detecting in an assay carboxylesterase activity or carboxylesterase and lipase activity (respectively) of a contaminating host cell protein in a sample comprising a recombinant protein of interest produced in a eukaryotic cell in cell culture, preferably wherein the recombinant protein is produced in a CHO cell and the at least one contaminating host cell protein is a CHO host cell protein (CHOP), wherein the hydrophilic substrate is a saturated unbranched-chain fatty acid (C6-C12) ester of 1-hydroxypyrene-3,6,8-trisulfonic acid or a salt thereof (substrate HPTS ester), and wherein the lipophilic substrate is a saturated unbranched-chain fatty acid (C6-C16) 4-MU ester.

[020a] In yet another aspect, the present disclosure relates to use of a hydrophilic substrate HPTS ester and a lipophilic substrate 4-MU ester as a substrate for detecting in an assay carboxylesterase and lipase activity of at least one contaminating host cell protein in a sample comprising a recombinant protein of interest produced in a eukaryotic cell in cell culture, preferably wherein the recombinant protein is produced in a CHO cell and the at least one contaminating host cell protein is a CHO host cell protein (CHOP),

wherein the hydrophilic substrate is a saturated unbranched-chain fatty acid (C6-C12) ester of 1-hydroxypyrene-3,6,8-trisulfonic acid or a salt thereof (substrate HPTS ester), and

wherein the lipophilic substrate is a saturated unbranched-chain fatty acid (C6-C16) 4-MU ester.

[021] In yet another aspect the disclosure relates to a use of a hydrophilic substrate HPTS ester as a substrate for detecting in an assay carboxylesterase activity of at least one contaminating host cell protein in a sample comprising a recombinant protein of interest produced in a eukaryotic cell in cell culture, preferably wherein the recombinant protein is produced in a CHO cell and the at least one contaminating host cell protein is a CHO host cell protein (CHOP), wherein the hydrophilic substrate is a saturated unbranched-chain fatty acid (C6-C12) ester of 1-hydroxypyrene-3,6,8-trisulfonic acid or a salt thereof (substrate HPTS ester).