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Publisher's version / Version de l'éditeur:

<https://doi.org/10.1016/j.jim.2010.09.027>

Journal of Immunological Methods, 362, 1-2, pp. 161-167, 2010-10-01

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Research paper

New ELISA approach based on coiled-coil interactions

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ARTICLE INFO

Article history:

Received 22 June 2010

Received in revised form 2 September 2010

Accepted 16 September 2010

Available online 1 October 2010

Keywords:

EGF

E/K coiled-coil interactions

ELISA test

ABSTRACT

The *de novo* designed heterodimeric E/K coiled-coil system has been previously demonstrated to be an excellent capture/dimerization system applicable to various needs in both biotechnology and pharmaceutical fields. Those include controlled protein dimerization, capture, purification and Western-blot detection. We here report the development of a new generation of ELISA test based on coiled-coil interactions for the direct quantitation of coil-tagged epidermal growth factor (EGF). The new approach was evaluated for its specificity, plate storability and reusability as well as for convenience when compared to commercially available systems. Our results show a similar affinity/sensitivity to standard capturing antibody-based ELISA systems and an improved affinity/sensitivity when compared to the commercially available Ni-NTA capture system. The E/K coiled-coil ELISA system was validated with respect to recovery, intra- and inter-assay variations. The practical working range was estimated to be between 5.2 and 34,000 pM. Furthermore, the storability and reusability of the plates was greater than the two aforementioned systems, suggesting that the E/K coiled-coil system is a good alternative to traditional tags such as poly-histidine for the development of ELISA tests aiming at quantitating coil-tagged proteins.

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1. Introduction

α -helical coiled-coil is an oligomerization motif being naturally present in a wide variety of proteins such as transcription factors, motor and viral fusion proteins (Burkhard et al., 2001). In fact, protein data base analysis revealed that approximately 10% of all proteins contain coiled-coil domains, therefore making it one of the most abundant motif found in proteins (Mewes et al., 2000; Walshaw and Woolfson, 2001). Coiled-coil motif results from the wrapping of two or more amphipathic α -helices around each other in a left handed supercoil fashion; the helices being aligned in either a parallel or anti-parallel manner (Lupas, 1996). Each peptide involved in

this domain possesses a characteristic signature, i.e. a heptad repeat denoted (abcdefg)_n, in which hydrophobic residues at the (a) and (d) positions pack in a characteristic 'knobs-into-holes' manner to form the hydrophobic core of the coiled-coil structure (Crick, 1953). The structural properties of coiled-coils have been extensively characterized by high-resolution crystallography and NMR-based studies. The influence of each residue of the heptads upon coiled-coil stoichiometry, stability as well as relative alignment of α -helices composing the motif (i.e., parallel or anti-parallel) are now well understood (Parry et al., 2008). This knowledge has opened the way to the design of *de novo* heterodimeric coiled-coils for protein engineering and biotechnological applications. Of particular interest, the *de novo* designed heterodimeric E/K coiled-coil system has been demonstrated to be a promising capture/dimerization tool for various applications (Tripet et al., 1996; Chao et al., 1998). Within the E/K coiled-coil complex, each peptide is composed of a distinct heptad that is repeated five times (Ecoil: [VSALEKE]₅;

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Kcoil: [VSALKEK]₅), in which hydrophobic residues at the (a) and (d) positions (V and L, respectively) are common to both peptides, while residues at positions (e) and (g) are distinct for each peptide (E or K). These residues further stabilize the E/K coiled-coil structure through electrostatic interactions while increasing specificity. Among the applications of the E/K peptides are the stable capture of fully bioactive proteins on biosensor (De Crescenzo et al., 2003b) and cell culture-compatible surfaces (Boucher et al., 2009) or virus pili (Le et al., 2009), the artificial dimerization of growth factors (Nakaji-Hirabayashi et al., 2009) or receptor ectodomains (De Crescenzo et al., 2004) for increased biological activities and the detection of coil-tagged protein by Western blot (Boucher et al., 2010). Furthermore, this coiled-coil system was demonstrated to be promising for the development of affinity chromatography strategies (Tripet et al., 1996; Chao et al., 1998).

We here report another application for the E/K system, namely, the development and the validation of a novel enzyme linked immunosorbent assay (ELISA) approach for the detection of coil-tagged proteins. In that endeavor, a chimeric protein corresponding to the epidermal growth factor (EGF) fused to both Ecoil and His tags at its N-terminus was used as a model protein. We compared the detection sensitivity of our novel ELISA approach that relied on coiled-coil mediated protein capture to those of standard ELISAs based on His tag- or antibody-mediated capture. Secondly, we evaluated the storability, reusability and regeneration of the system and found many advantages when compared to current commercial ones. The E/K coiled-coil system was also shown to be efficient for the determination of coil-tagged EGF concentration being present in complex mixtures such as cell culture medium. Altogether, our results demonstrated that the novel E/K coiled-coil-based ELISA approach is more efficient than commercially available affinity-based systems at a lower cost. Furthermore, the newly developed system showed improved storability and enhanced reusability when compared to both Ni-NTA systems and standard ELISAs.

Fig. 1. Dose–response curves corresponding to the detection of (♦) untagged human EGF and (■) Ecoil-(His)₈-EGF incubated on capture antibody-coated bare plates. Optical density recorded at 450 nm was corrected at 540 nm. Results are the means $\pm$ standard deviation of five plates.

2. Materials and methods

2.1. Material and reagents

Aminated microplates (96 Well Clear Polystyrene Amine Surface Stripwell Microplate, Corning catalog #2388) were purchased from Fisher Scientific Co. (Ottawa, ON). Bare (standard) microplates (96 Well Clear Polystyrene Stripwell Microplates, Costar catalog #2592) were obtained from R&D Systems (Minneapolis, MN). Ni-NTA microplates (Ni-NTA HisSorb plate, Qiagen catalog #35061) were purchased from Qiagen (Mississauga, ON). Dulbecco's Phosphate Buffered Saline (modified PBS, without calcium chloride and magnesium chloride), Tween 20, cysteine (99 + % purity), guanidine hydrochloride (Gnd-HCl) and sodium chloride (99.99% purity) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON). Sulfuric acid (98% purity) was purchased from VWR International Inc (Mont-Royal, QC). Succinimidyl 6-[30-(2-pyridyldithio)-propionamido]hexanoate (LC-SPDP, 95 + % purity) was obtained from Pierce Biotechnology, Inc. (Rockford, IL). MilliQ quality water (18.2 M Ω cm; total organic compounds (TOC) = 4 ppb) was generated with a Millipore Gradient A 10 purification system. Cysteine-tagged Kcoil peptides (De Crescenzo et al., 2003a) were synthesized by the peptide facility at University of Colorado (Denver, CO). Commercially available DuoSet ELISA kit containing mouse anti-human EGF antibody (capture antibody), biotinylated goat anti-human EGF antibody (detection antibody), streptavidin-horseradish peroxidase (streptavidin-HRP), bovine serum albumin (BSA), substrate solution (hydrogen peroxide/tetramethylbenzidine) and untagged human EGF were purchased from R&D Systems (Minneapolis, MN).

2.2. Plasmid

The pTT5-Ecoil-EGF plasmid coding for Ecoil-(His)₈-EGF, a recombinant protein corresponding to human EGF N-terminally fused to the Ecoil (5 heptad repeats) and to an (His)₈ affinity tag (MW = 12,711 Da), was constructed by in-frame ligation of Ecoil, (His)₈ tag and human EGF cDNA sequences as previously described (Boucher et al., 2008).

2.3. Ecoil-(His)₈-EGF production and purification

Ecoil-(His)₈-EGF was produced in HEK 293-6E cells and purified by immobilized metal ion affinity chromatography (IMAC) as previously reported (Boucher et al., 2008). Protein concentration was determined by Bradford assay and analyzed by SDS-PAGE. Purified tagged EGF was then aliquoted and stored at -80°C until use. Note that, within this chimeric protein, the EGF moiety was demonstrated to be as active as untagged EGF in in vitro cell assay (Boucher et al., 2008).

For small-scale Ecoil-(His)₈-EGF production, HEK293-6E cells were seeded in 6-well cell culture plates (1.67×10^6 cells/ml in 1.8 mL medium). 2 μg of pTT5-Ecoil-EGF plasmid were diluted in 100 μL of F17 medium to which 100 μL of F17 medium containing 4 μg of 25 kDa linear polyethylenimine was added. The mixture was immediately vortexed and incubated for 15 min at room temperature prior to cell addition. Cells were fed 24 h post-transfection (hpt) with 0.5% TN1 peptone (w/v, final) (Pham et al., 2005). Following harvest at different

Fig. 2. Schematic illustration of Ecoil-(His)₈-EGF capture onto Kcoil-coated wells. Thiol-reactive LC-SPDP linker attached on aminated well allowed for the covalent grafting of cysteine-tagged Kcoil peptides. Remaining active linkers were blocked using cysteine. Ecoil-(His)₈-EGF were captured to the well surface via reversible E/K coiled-coil interactions.

time points (up to 120 hpt), the medium was clarified by centrifugation (3500×g, 10 min). Ecoil-(His)₈-EGF present in the supernatant was then quantified by ELISA (see below).

2.4. Plate preparation

2.4.1. Capture antibody coating on standard microplates

Capture antibody was diluted to 4.0 µg/mL in 10 mM PBS without carrier protein. 100 µL of the dilute solution were transferred to the bare microplate wells and allowed to incubate overnight at room temperature (RT). The plates were then washed three times with 200 µL of wash buffer, consisting of 10 mM PBS, 0.05% Tween 20 (PBS-T). In order to block the plates, 200 µL of PBS containing 1% bovine serum albumin (PBS-BSA) was added into each well and then incubated for 1 h at RT. The plates were washed three times with PBS-T.

2.4.2. Kcoil peptides grafting on aminated microplates

Kcoil layers were generated on aminated plates via LC-SPDP linker by adapting the protocol from Boucher et al. (Boucher et al., 2009). Briefly, 100 µL of 2 mM LC-SPDP in 10 mM phosphate buffer (10% DMSO) were added to each well for 1 h at RT. The plates were rinsed four times using 200 µL of MilliQ water. Then, the thiol-reactive wells were incubated with 100 µL of 10 µM cysteine-tagged Kcoil peptides (5 heptad repeats) in 10 mM PBS for 2 h at RT. The plates were rinsed two times using 200 µL of 10 mM PBS and finally four times using 200 µL of MilliQ water. Unreacted LC-SPDP sites were blocked using 100 µL of 50 mM cysteine solution (1 M NaCl in 0.1 M sodium acetate, pH 4.0) followed by rinsing with MilliQ water. The Kcoil-coated plates dried in air can be stored at 4 °C until use.

Table 1

Impact of BSA pretreatment on the non-specific adsorption of untagged EGF. Optical densities were obtained by incubating 34 nM of untagged EGF on cysteine- and Kcoil-functionalized plates. Results correspond to the mean ± standard deviation (*n* = 4).

BSA blocking treatment	Covalently grafted layers	
	Cysteine	Kcoil peptide
–	1.031 ± 0.042	0.014 ± 0.003
+	0.158 ± 0.002	0.006 ± 0.002

2.4.3. Ni-NTA microplates

Ni-NTA-coated plates were used without any pretreatment, as recommended by the manufacturer.

2.5. Assay procedure

In the subsequent ELISA procedure, samples or standards refer to untagged EGF for capture antibody-coated plates and Ecoil-(His)₈-EGF for Kcoil-coated or Ni-NTA-coated plates.

100 µL of sample or standard diluted in PBS-BSA via direct dilutions were added into the corresponding wells. Plates were allowed to incubate for 1 h at RT. After washing the plates three times with PBS-T, 100 µL of detection antibody (50 ng/mL in PBS-BSA) were added to the plates followed by incubation for 1 h at RT. After a second washing with PBS-T, 100 µL of streptavidin-HRP (diluted 200 times with the PBS-BSA) was added to the plates and the solution was incubated for 20 min at RT. Following final washing with PBS-T, the reaction was revealed with 100 µL of substrate solution (1:1 mixture of hydrogen peroxide/tetramethylbenzidine). After an incubation time of 17 min in the dark at RT, the colorimetric reaction was stopped by adding 50 µL of 2 N H₂SO₄. The optical density (OD) at 450 nm and 540 nm (for correction) was measured using an ELISA plate reader (Victor³ V Multilabel Counter, PerkinElmer Inc., Woodbridge, ON).

Fig. 3. Dose-response curves corresponding to Ecoil-(His)₈-EGF incubation on (■) capture antibody-coated bare plates, (▲) Kcoil-coated aminated plates and (●) Ni-NTA plates. Optical density recorded at 450 nm was corrected at 540 nm. Results are the means ± standard deviation of five plates.

Fig. 4. Dose-response curves corresponding to Ecoil-(His)₈-EGF incubation (▲) with or (◆) without BSA pretreatment on Kcoil-coated aminated plates. Results are the means $\pm$ standard deviation of four wells.

2.6. Regeneration of Kcoil-coated microplates

The detachment of the coil-tagged EGF from the well surface was performed by covering each well with 200 μ L of 5 M guanidine hydrochloride solution in a plate shaker for 5 min, followed by rinses using 200 μ L of 10 mM PBS and finally MilliQ water. This cleaning cycle was repeated 10 times.

2.7. Cut-off index and working range determination

The cut-off index (COI) value was determined by an ELISA sensitivity test. Briefly, untagged EGF (34 nM) was incubated in the Kcoil-coated wells of modified plates and ELISA test was repeated 5 times. The value of the blank average OD (0.014) added to the value of the OD standard deviation multiplied by two (0.003×2) was determined to be the cut-off index value ($\text{COI} = 0.02$). Above this value, ODs are considered to be significant. The working range was determined by triplicate dilutions of recombinant Ecoil-(His)₈-EGF at concentrations ranging from 0.6 pM to 34,000 pM. The final working range was determined by combined evaluation of plotting 'measured over mean' as a function of concentration. Intra- and inter-assay variations were determined in order to verify the reproducibility and specificity of our assay. Intra- and inter-assay variations were determined by dilutions of the Ecoil-(His)₈-EGF and triplicate testing on either the same plate or different plates, according to the ELISA conditions described above.

2.8. Statistical analysis

Values are given as mean value $\pm$ standard deviation (s.d.). The data were expressed as means $\pm$ s.d. and analyzed using Statistica 9.0 (StatSoft, Inc., Tulsa, USA). Statistical significance was determined by one-way ANOVA and main effect ANOVA followed by Tukey HSD post-hoc test. Differences were considered significant for $p < 0.05$.

3. Results and discussion

While small affinity tags such as poly-histidine tails are now broadly used for the purification and detection of recombinant proteins, *de novo* engineered coil peptide tags have also proven to be promising for affinity purification and excellent in many other biotechnology applications including Western-blot detection (Boucher et al., 2010) protein capture (De Crescenzo et al., 2003b; Boucher et al., 2009) as well as controlled protein dimerization (De Crescenzo et al., 2004; Noirclerc-Savoye et al., 2005). While coil tags can be added to peptides via chemical reaction (Tripet et al., 2003), the production of more-complex coil-tagged proteins in bacteria (Le et al., 2009) and mammalian cells has also been reported (De Crescenzo et al., 2003b; Boucher et al., 2008). In that endeavor, the development of efficient expression vectors containing cDNA coding for signal peptide (to ease protein secretion) and cDNA coding for coil tag have already been designed and validated for the production of fully bioactive complex proteins (De Crescenzo et al., 2003b). The present work further extends the use of this new tag system to the development of ELISA test aiming at quantitating coil-tagged recombinant proteins that are produced by these new expression platforms. In that endeavor, Ecoil-(His)₈-EGF, expressed by transiently transfected HEK293-6E cells was used as a model protein.

More specifically, antibody/antigen, coiled-coil and His/Ni-NTA interactions have been explored to capture Ecoil-(His)₈-EGF proteins onto anti-EGF antibody, Kcoil and Ni-NTA-coated ELISA plates, respectively. The amount of EGF protein attached to the wells via those three different approaches was then evaluated by applying a standard ELISA test, i.e. addition of biotinylated anti-EGF antibody followed by the attachment of streptavidin-HRP and colorimetric reaction. In order to compare the different approaches, detection antibody, streptavidin-HRP and HRP substrate were added to the wells at the same concentration and the colorimetric oxidation reaction was allowed to proceed for the same duration in each case (17 min).

First, antibody-mediated capture of Ecoil-(His)₈-EGF was evaluated using conventional ELISA technique with antibody-

Table 2

Intra- and inter-assay coefficients (optical density variation in %) for Ecoil-(His)₈-EGF captured on antibody-coated, Kcoil-coated and Ni-NTA-coated plates. Results are means of five plates.

		Concentration (pM)								
		34,000.0	11,333.3	3777.8	1259.3	419.8	139.9	46.6	15.5	5.2
Capture antibody	Intra	1.9	1.9	1.8	1.7	1.9	3.5	3.1	4.4	4.4
	Inter	1.6	1.9	1.6	1.1	2.1	3.1	7.0	6.2	12.1
Kcoil	Intra	2.3	2.8	2.7	3.3	3.3	3.7	5.4	4.1	9.3
	Inter	1.2	1.2	2.2	3.4	3.9	6.2	6.2	5.0	10.9
Ni-NTA	Intra	2.1	3.0	2.2	2.9	3.5	5.2	7.8	8.1	10.3
	Inter	3.7	1.6	1.0	2.2	4.5	8.4	8.6	11.4	13.8

Fig. 5. Dose–response curves corresponding to Ecoil-(His)₈-EGF incubation on fresh (▲) and aged (◆) Kcoil-grafted aminated plates. Fresh plates were generated at least 2 h before Ecoil-(His)₈-EGF loading. Artificially aged plates were generated 4 weeks before use and stored in a dried state at 4 °C. Optical density recorded at 450 nm was corrected at 540 nm. Results are the means ± standard deviation of five and two plates for freshly made and aged plates, respectively.

coated standard plates and compared to that of untagged EGF. As seen in Fig. 1, the concentration curves corresponding to both tagged and untagged EGF overlapped, thus indicating that neither Ecoil nor His tag impacted Ecoil-(His)₈-EGF capture by anti-EGF antibody. This result is in excellent agreement with our previous study that had demonstrated that both Ecoil and His tags did not affect Ecoil-(His)₈-EGF bioactivity as deduced from *in vitro* cell assays (Boucher et al., 2008).

Secondly, Kcoil-coated microplates were generated by adapting the optimized grafting conditions we previously described (Boucher et al., 2009). To do so, commercially available aminated plates were reacted with bifunctional LC-SPDP linker to create a stable amide bond. The other extremity of LC-SPDP linking agent was then reacted with the thiol group of the cysteine-terminated Kcoil peptide to form a covalent disulfide bond. After Kcoil grafting, cysteine was used to block the unreacted thiol-reactive groups of the LC-SPDP in order to prevent the subsequent attachment of molecules

Fig. 6. Dose–response curves corresponding to Ecoil-(His)₈-EGF incubation on (▲) fresh and (■) regenerated Kcoil-coated aminated plates. Regeneration of Kcoil plates was performed using guanidine hydrochloride to detach captured Ecoil-(His)₈-EGF, as described in the *Material and Methods* section. Remaining Ecoil-(His)₈-EGF after washing protocol is shown as (●). Optical density recorded at 450 nm was corrected at 540 nm. Each data point corresponds to the mean ± standard deviation of four wells.

containing thiol groups. Ecoil-tagged EGF proteins were then captured onto Kcoil-modified surface via reversible coiled-coil interactions by simple incubation (Fig. 2).

The specificity of Kcoil-coated plates towards Ecoil-(His)₈-EGF was then evaluated by incubating untagged EGF on aminated wells that had been coated with Kcoil peptide or with cysteine only as a reference. A concentration of 34 nM of untagged EGF was incubated in the modified wells. Our results showed that a higher optical density (OD) value was obtained on cysteine-covered wells (1.031) when compared to Kcoil-covered wells (0.014) (see Table 1). This result indicated that a large amount of untagged EGF peptide could adsorb non-specifically on cysteine-grafted wells whereas Kcoil grafting at the surface of the wells was efficient to block non-specific EGF adsorption. This further suggests that Kcoil grafting, using our experimental protocol, resulted in complete and very efficient microplate surface coverage. This conclusion is further supported by the fact that Kcoil-functionalized microplate pretreatment using bovine serum albumin (BSA) as blocking agent did not drastically reduce the non-specific adsorption of untagged EGF since OD only decreased from 0.014 to 0.006 (Table 1). In stark contrast, the same BSA pretreatment significantly reduced untagged EGF non-specific adsorption onto cysteine-covered wells (OD decrease from 1.031 to 0.158, Table 1).

In order to test the efficiency of our coiled-coil based ELISA system, we compared dose–response standard curves corresponding to various concentrations of Ecoil-(His)₈-EGF being incubated onto antibody-coated, Kcoil-grafted and commercially available Ni-NTA plates. As seen in Fig. 3, the standard dose–response curves shifted to higher concentration values for the tag-specific plates compared to capture antibody-coated plates by a factor 4 and 10 for Kcoil- and Ni-NTA-coated plates, respectively (Fig. 3). These shifts corresponded to apparent thermodynamic dissociation constants, K_D , equal to 70, 185 and 675 pM for antibody/Ecoil-(His)₈-EGF, Kcoil/Ecoil-(His)₈-EGF and Ni-NTA/Ecoil-(His)₈-EGF interactions, respectively, as deduced from fitting Fig. 3 data with a simple interaction model (Langmuir isotherm). The apparent K_D corresponding to Kcoil/Ecoil-(His)₈-EGF interactions, as determined by ELISA (i.e. 185 pM) was in good agreement with that determined for Ecoil/Kcoil interactions using a surface plasmon resonance biosensing approach (K_D = 63 pM) (De Crescenzo et al., 2003a). Altogether, our results thus indicate that the Kcoil system is more efficient than the commercially available Ni-NTA affinity-based system for Ecoil-(His)₈-EGF capture. The influence of BSA pretreatment prior to Ecoil-(His)₈-EGF incubation on Kcoil-coated plates was also evaluated. As shown in Fig. 4, superimposable dose–response curves were obtained with or without BSA pretreatment, confirming that the latter is not required for Kcoil plate usage as previously deduced (Table 1), as long as BSA is present in the PBS solution used for diluting samples.

To determine the practical working range of the new system (i.e., without BSA pretreatment), we performed OD reading for various dilutions of Ecoil-(His)₈-EGF (from 0.6 to 34,000 pM). Intra-assay OD variations of less than 10% were obtained within the 5.2-to-34,000 pM range (Table 2), which thus defines the practical working range of our coiled-coil-based ELISA. In addition, inter-assay variations in that working range were also acceptable ($\leq 10\%$) thus making our system as efficient as commercially available ones (Table 2).

Fig. 7. Ecoil-(His)₈-EGF quantification in transiently transfected HEK293-6E cell culture medium after. Culture media were collected at different times (24 to 120 h) post-transfection and Ecoil-(His)₈-EGF concentrations were determined using coiled-coil-based (▲) and standard (■) ELISA (1/100 and 1/500 sample dilution respectively).

Kcoil-coated microplate storage stability and reusability were then assayed. Freshly made plates and aged plates that had been stored for 4 weeks at 4 °C gave comparable dose-response curves for Ecoil-(His)₈-EGF detection, thus indicating that our Kcoil coating was stable, and thus suitable for commercial purposes (Fig. 5). For Kcoil-coated plate reusability, guanidine hydrochloride (Gnd-HCl, 5 M), a strong chaotropic agent, was used to promote Ecoil-(His)₈-EGF/Kcoil complex dissociation. Gnd-HCl was selected based on our previous results that indicated that coiled-coil complexes could be disrupted with such denaturing agent (De Crescenzo et al., 2003b). Ecoil-(His)₈-EGF amounts remaining in the wells were then revealed with the same detection antibody as that we used in previous experiments, since its binding to EGF is not dependent upon the conformation of the latter (Gnd-HCl treatment might have unfolded the EGF moiety of the fusion protein). As seen in Fig. 6, a complete detachment of captured Ecoil-(His)₈-EGF was obtained following ten Gnd-HCl washing cycles when the tagged growth factor had been incubated at concentrations lower than 420 pM (i.e. approximately 70% well saturation).

Higher amounts of captured Ecoil-(His)₈-EGF resulting from incubations at concentrations higher than 420 pM, were not completely stripped with such a treatment; increasing the number of washing cycles did not further enhance Ecoil-(His)₈-EGF removal (data not shown), indicating a potential limitation of the method. Following Kcoil microplate stripping, its reusability was assessed by reloading fresh Ecoil-(His)₈-EGF in the wells for which complete removal had been attained. As shown in Fig. 6, standard dose-response curves corresponding to stripped and fresh plates overlapped, thus unambiguously demonstrating that our washing protocol did not affect the capture properties of the Kcoil microplates. Our Kcoil-coated ELISA plates are reusable when moderate amounts of Ecoil-(His)₈-EGF (corresponding less than 70% well saturation) are captured; within that range, i.e. $0 < [\text{Ecoil}-(\text{His})_8\text{-EGF}] < 420$ pM, the calibration curve was observed to be linear.

We then tested the applicability of our coiled-coil-based ELISA system for the quantification of Ecoil-(His)₈-EGF in cell culture medium. To do so, small-scale transient transfections of

HEK293-6E cells with plasmid encoding Ecoil-(His)₈-EGF were performed in 6-well culture plates. Cell culture supernatants were harvested everyday for 5 days post-transfection and Ecoil-(His)₈-EGF concentrations were determined using our coiled-coil- and antibody-based standard ELISA tests. As seen in Fig. 7, both ELISA tests gave identical results, hence unambiguously demonstrating that our coiled-coil-based approach is adequate for Ecoil-(His)₈-EGF quantification in complex mixtures such as cell culture medium.

4. Conclusion

We have demonstrated the validity of a coiled-coil-based ELISA approach for coil-tagged EGF detection. We have also highlighted its advantages such as plate stability upon storage and reusability and its improved affinity/sensitivity when compared to that of commercially available Ni-NTA-based ELISA. The unique features of the new assay make it ideally suited for Ecoil-tagged recombinant protein detection and quantification. Furthermore, this novel assay has the potential to be optimized for coil-tagged protein interaction studies and high throughput protein quantification.

Acknowledgement

This work was supported by the Canada Research Chairs on Protein-enhanced Biomaterials (GDC), on Applied Metabolic Engineering (MJ), by the Natural Sciences and Engineering Research Council of Canada (GDC, MJ) and by the Groupe de Recherche en Sciences et Technologies Biomédicales (GRSTB) through a post-doctoral fellowship to BL.

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