Transient expression in HEK 293 cells – an alternative to *E. coli* for the production of secreted and intracellular mammalian proteins.

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Summary

Transient transfection of human embryonic kidney cells (HEK 293) enables the rapid and affordable lab-scale production of recombinant proteins. In this chapter protocols for the expression and purification of both secreted and intracellular proteins using transient expression in HEK 293 cells are described.

1 Introduction

High quality, pure proteins are important reagents for a wide variety of applications such as biochemical assays, protein-based therapeutics and protein crystallography. *E. coli* remains the most commonly used expression host for producing recombinant proteins for research purposes, for example structural studies, due to its ease of use and relatively low cost. However, production of recombinant proteins in high yield from *E. coli* can be challenging due to low expression levels and poor solubility. This is particularly the case for mammalian proteins. Although expression of many human intracellular proteins has been tried in *E. coli*, about 65 % are either not expressed or expressed insolubly (1). These problems may be overcome by using mammalian cells for protein production as these express the necessary chaperones for correct folding and contain the machinery for adding post-translational modifications (PTMs). Mammalian cells also contain small molecules and cofactors which may be required for protein expression or complex formation.

Two mammalian cell lines are routinely used for the production of recombinant proteins, Chinese hamster ovary (CHO) and human embryonic kidney (HEK 293) cells. Of these, HEK 293 cells have become the mammalian cell line of choice for lab-scale protein production due to their ease of culture and high transfection efficiency (2). A useful variant of HEK cells is the 293T cell line which expresses the SV40 large T antigen. Expression vectors containing the SV40 origin of replication are episomally amplified within the 293T cells, which increases the plasmid copy number per cell and can lead to higher levels of transient expression (3). A further variant of the HEK cell line is the FreeStyle™ HEK 293F cell line (Life Technologies, UK) in which the HEK 293 cells are adapted to suspension growth in FreeStyle™ 293 expression

medium. The medium is designed to support high-density growth and has the advantage of allowing transfection without the need to change medium.

The use of the inexpensive cationic polymer, polyethylenimine (4-5) as the DNA condensing reagent has meant that large scale transient transfection of HEK 293 cells has become economically feasible and is routinely used for the production of secreted and cell surface glycoproteins (reviewed by Aricescu and Owens (6)).

In contrast to their use with secreted proteins, mammalian cells have not been used routinely for the production of intracellular proteins due to the relatively low levels of expression compared with insect or bacterial systems. However, by using highly selective purification methods, e.g. FLAG® tag (7) or Halo® tag (8), it is possible to achieve useful yields of intracellular proteins. Again, transient expression in HEK 293 cells offers a way of rapidly assessing the protein yield and quality. Subsequent production of stable cell lines, typically by co-selection, may be required to sustain and improve the production levels of a particular product.

In this chapter, protocols for the production of both secreted and intracellular proteins by transient transfection of HEK 293 cells are described. The methods are exemplified by reference to the production of the secreted protein, human serum amyloid P component (SAP) and the intracellular proteins, human brain specific protein kinase C isoform protein kinase M zeta (PKMζ) and human histone deacetylase 3 (HDAC3) in complex with its activation domain from the SMRT co-repressor (SMRT-DAD).

SAP is a plasma glycoprotein (9) which participates in the innate human immune system but also plays a role in the molecular pathology of diseases such as amyloidosis and amyloid associated diseases such as Alzheimer's and Type II diabetes (10). SAP is of increasing clinical relevance

as radio-labelled SAP is used for identifying sites of amyloid deposition (11), whilst drug development programs attempting to deplete serum levels (for treatment of amyloidosis) and also administer protein (for treatment of fibrosis) are currently underway (12-13). SAP contains an N-glycan and a disulphide bridge and is representative of proteins with these modifications.

PKMζ is a neuron-specific isoform of atypical protein kinase C (aPKC) that lacks the normal N-terminal regulatory region and therefore comprises just a kinase catalytic domain (14). In vivo phosphorylation of PKMζ by PDK1 converts PKMζ into a conformation with high constitutive activity (15). Although there is controversy as to the extent and nature of its role, PKMζ has been implicated in both memory (16) and pain (17). PKMζ contains 5 cysteines (with the potential for disulphide bridge formation) and is activated via phosphorylation.

HDAC3 is a class I histone deacetylase (HDAC) that is involved in transcriptional regulation (18). Like the other class I HDACs, HDAC3 requires recruitment to its cognate co-repressor protein (SMRT) to have full enzymatic activity (19). HDACs are important therapeutic targets for the treatment of cancer (20), and are involved in other diseases such as Alzheimer's and HIV (21-22). HDAC3 and its activation domain from the SMRT co-repressor (SMRT-DAD) do not interact when expressed in bacterial cells but require expression in higher eukaryotes to form a complex. The HDAC3:SMRT-DAD complex is phosphorylated in the C-terminal region of HDAC3 and also acetylated (as determined by mass spectrometry). The structure of the HDAC3:SMRT-DAD complex revealed the presence of an Ins(1,4,5,6)P_4 molecule at the interface between HDAC3 and SMRT which is required for complex formation and activation (23).

To show the benefit of using mammalian rather than bacterial cells to express human proteins, SAP and PKMζ were tested for expression in both *E. coli* and HEK 293 cells (24-26). In Figure 1 it can be seen that SAP in a vector containing a signal sequence is expressed and secreted using HEK cells (Figure 1, lanes 1 and 2), but not in *E. coli* (lanes 3 and 4). Without the signal sequence, some SAP is accumulated in the cells using HEK cell expression (lane 6), but there is no expression in *E. coli* (lanes 7 and 8). The band in lane 6 migrates lower than those in lanes 1 and 2 as no post-translational modification has taken place. For PKMζ, expression can be seen in the cells and in the soluble extraction for both HEK cells and *E. coli* (lanes 9 to 12), however expression levels are higher using HEK cells (lanes 9 and 10).

2 Materials

2.1 Protein expression using HEK 293T cells

1. HEK 293T cells (ATCC no. CRL-1573 – LGC Standards, UK).
2. Dulbecco's modified Eagles medium (DMEM) (Life Technologies, UK).
3. Foetal calf serum (FCS) (Biosera, France).
4. Non-essential amino acids (1:100) (Life Technologies, UK).
5. L-Glutamine (Sigma-Aldrich, UK).
6. T175 tissue culture flask (Greiner Bio-One, UK).
7. Plasmid DNA: The gene of interest needs to be contained in a vector compatible with mammalian expression systems (*See* Note 1).
8. Polyethyleneimine (PEI) (25 kDa branched PEI – Sigma-Aldrich, UK). Prepare a 100 mg/ml stock solution in water before diluting to 1 mg/ml. Neutralise the solution with HCl, filter sterilise and store at -20°C in aliquots.

9. Kifunensine (Toronto Research Chemicals, Canada).
10. Expanded surface roller bottles (Greiner Bio-One, UK).
11. PBS: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4 (tablets from Sigma-Aldrich, UK).

2.2 Protein expression using HEK 293F cells

1. FreeStyle™ HEK 293F cells (Life Technologies, UK).
2. Gibco® FreeStyle™ 293 expression medium (Life Technologies, UK).
3. 250 ml Erlenmeyer flask with vent cap (Corning, UK).
4. Vent cap roller bottle (Corning, UK).
5. Plasmid DNA: The gene of interest needs to be contained in a vector compatible with mammalian expression systems (*See* Note 1).
6. Polyethyleneimine (PEI) (25 kDa branched PEI – Sigma-Aldrich, UK). Prepare a 0.5 mg/ml stock solution in water. Neutralise the solution with HCl, filter sterilise and store at -20°C in aliquots.
7. Kifunensine (Toronto Research Chemicals, Canada).
8. Dulbecco's phosphate buffered saline (Sigma-Aldrich, UK).

2.3 Purification of secreted proteins using a His_6 tag

1. Äkta purification system such as Äkta Xpress (GE Healthcare Life Sciences, UK).
2. HiLoad 16/600 Superdex S75 or S200 (GE Healthcare Life Sciences, UK).
3. Gel Filtration Buffer: 20 mM Tris-HCl, 200 mM NaCl, pH 8.0.
4. Nickel Wash Buffer: 50 mM Tris-HCl, 500 mM NaCl, 30 mM imidazole, pH 8.0.

5. Nickel Elution Buffer: 50 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 8.0.
6. 96 deep well plate (Greiner Bio-One, UK).
7. 5 ml HisTrap FF column (GE Healthcare Life Sciences, UK).

2.4 Purification of intracellular proteins using a 3xFLAG® tag

1. Flag Lysis Buffer: 100 mM potassium acetate, 50 mM Tris base pH 7.5, 5 % (v/v) glycerol, 0.3 % Triton X-100, Roche complete protease inhibitor tablet (Roche, UK).
2. Flag Wash Buffer 1: 100 mM potassium acetate, 50 mM Tris base pH 7.5, 5 % (v/v) glycerol, 0.3 % Triton X-100.
3. Flag Wash Buffer 2: 300 mM potassium acetate, 50 mM Tris base pH 7.5, 5 % (v/v) glycerol.
4. Flag Cleavage Buffer: 50 mM potassium acetate, 50 mM Tris base pH 7.5, 5 % (v/v) glycerol, 0.5 mM tris (2-carboxyethyl) phosphine (TCEP).
5. Anti-FLAG® M2 resin (Sigma-Aldrich, UK).
6. Flag Equilibration Buffer: 100 mM potassium acetate, 50 mM Tris base pH 7.5.
7. His-TEV (Tobacco etch virus) protease solution. A plasmid for the expression of His-tagged TEV protease using *E. coli* is available from Addgene, USA (www.addgene.org).
8. Amicon Ultra Centrifugal filter (Millipore, UK).
9. HiLoad 10/300 Superdex S75 or S200 (GE Healthcare Life Sciences, UK).
10. Gel Filtration Buffer: 50 mM potassium acetate, 25 mM Tris base pH 7.5, 0.5 mM TCEP.

3 Methods

3.1 Protein expression using attached HEK 293T cells

Depending on the scale of expression required and the equipment available, methods for both T175 static flasks and roller bottles are described below.

3.1.1 Medium scale using T175 flasks

1. All cell manipulations are carried out in a Class 2 laminar flow hood.
2. Seed HEK 293T cells at 7.5 x 10^5 cells/ml in 5 ml so that the cells are ~80 % confluent after 24 hours. Make up to 45 ml with DMEM containing 2 % FCS, 1 x non-essential amino acids and 1 mM glutamine.
3. Incubate the cells at 37°C in a 5 % CO_2/95 % air atmosphere for 24 hours.
4. Mix 87.5 µl plasmid DNA (*See* Note 2) with 2.6 ml of DMEM supplemented with 1 x non-essential amino acids and 1 mM glutamine.
5. In a separate vessel, mix 154 µl 1 mg/ml PEI with 2.6 ml of DMEM containing 1 x non-essential amino acids and 1 mM glutamine. Add this to the DNA cocktail made in step 4 and mix thoroughly.
6. Incubate at room temperature for 10 minutes (*See* Note 3).
7. Remove the supernatant from the T175 flask of confluent HEK 293T cells.
8. Add the transfection cocktail made in steps 4-6 to the cells.
9. Top up the flask with 40 ml of DMEM containing 2 % FCS, 1 x non-essential amino acids and 1 mM glutamine.
10. If control of glycosylation is required, add 45 µl of 1 mg/ml kifunensine to the T175 flask (*See* Note 4).

11. Incubate the flask at 37°C in a 5 % CO_2/95 % air atmosphere for 3 days at which point the phenol red pH indicator in the DMEM should start to change colour to orange.
12. To harvest a secreted protein: Collect the supernatant (which contains the protein), centrifuge at 6,000*g* for 15 minutes to remove any detached cells, and filter through a 0.22 μm bottle top filter before storing at 4°C.
13. To harvest an intracellular protein: Remove the supernatant and discard before freezing the T175 flask at -80°C.

3.1.2 Large scale using roller bottles

1. Each roller bottle contains 250 ml of culture so 4 roller bottles are needed per litre of culture.
2. Seed HEK 293T cells at around 7.5 x 10^5 cells/ml in 20 ml into each roller bottle (*See* Note 5) and add 250 ml DMEM containing 2 % FCS, 1 x non-essential amino acids and 1 mM glutamine.
3. Incubate the roller bottle at 37°C for 4 days with the bottle rotating at 30 rpm (*See* Note 6). After this time, the cells should be ~80 % confluent.
4. Remove the spent media from the roller bottle and replace with 200 ml DMEM containing 2 % FCS, 1 x non-essential amino acids and 1 mM glutamine. Return the roller bottle to the incubator.
5. Mix 0.5 mg of plasmid DNA (*See* Note 2) with 25 ml of DMEM with 1 x non-essential amino acids and 1 mM glutamine.

6. In a separate vessel, mix 875 µl of 1 mg/ml PEI with 25 ml of DMEM containing 1 x essential amino acids and 1 mM glutamine. Add this to the DNA cocktail from step 5 and mix thoroughly.
7. Incubate at room temperature for 10 minutes (*See* Note 3).
8. Add the transfection cocktail made in steps 5-7 to the roller bottle.
9. Top up the flask with 200 ml of DMEM containing 2 % FCS, 1 x non-essential amino acids and 1 mM glutamine.
10. If control of glycosylation is required, add 0.25 ml of 1 mg/ml kifunensine to the roller bottle (*See* Note 4).
11. Incubate the roller bottle at 37°C with the bottle rotating at 30 rpm (*See* Note 6) for 3-6 days. The point of harvest is determined by the phenol red pH indicator in the DMEM starting to change colour to orange.
12. To harvest a secreted protein: Collect the supernatant (which contains the protein), centrifuge at 6,000*g* for 15 minutes to remove any detached cells, and filter through a 0.22 µm bottle top filter before storing at 4°C.
13. To harvest an intracellular protein: Remove the supernatant and discard. Detach cells from the roller bottle by shaking and harvest by centrifugation at 6,000*g* for 15 minutes. Wash the roller bottle in 125 ml PBS and use this solution to resuspend the cell pellet, thus washing the cells to remove any remaining media. Centrifuge for a further 15 minutes at 6,000*g* and freeze the resulting pellet at -80°C.

3.2 Protein expression using suspension HEK 293F cells

Depending on the scale of expression required and the equipment available, methods for both 250 ml Erlenmeyer flasks and roller bottles are described below. For co-transfections of two or more plasmids, the total amount of DNA used must be as indicated in the protocols below.

3.2.1 Medium scale using 250 ml flasks

1. 250 ml flasks will support between 30 and 100 ml culture. For transfection volumes greater than 30 ml the protocol can be scaled accordingly.
2. All cell manipulations are carried out in a Class 2 laminar flow hood.
3. Seed cells at 3.5 x 10^5 cells/ml in to a 250 ml flask with a final volume of 30 ml.
4. Incubate flask at 37°C in a 5 % CO_2/95 % air atmosphere with the bottle rotating at 120 rpm for 3 days until the cells reach a density of > 2 x 10^6 cells/ml.
5. Dilute 30 μg plasmid DNA (total) (*See* Note 2) in 3 ml PBS and vortex briefly.
6. Add 120 μL of 0.5 mg/ml PEI to the diluted DNA and vortex briefly.
7. Incubate at room temperature for 20 minutes.
8. Add the PBS, DNA and PEI cocktail to 27 ml cells at 1 x 10^6 cells/ml final concentration.
9. If control of glycosylation is required, add 30 μl of 1 mg/ml kifunensine to the flask (*See* Note 4).
10. Incubate flask at 37°C in a 5 % CO_2/95 % air atmosphere for 48 hours.
11. To harvest protein: centrifuge cells at 6,000*g* for 5 minutes. For intracellular protein retain cells and store at -80°C and for secreted protein retain supernatant and filter through a 0.22 μm bottle top filter before storing at 4°C.

3.2.2 Large scale using roller bottles

1. Each roller bottle contains 300 ml of culture so 4 roller bottles are needed for 1.2 l of culture. Roller bottles will support a minimum volume of 150 ml and a maximum volume of 300 ml, for volumes less than 300 ml the protocol can be scaled accordingly.
2. Seed cells at 3.5 x 10^5 cells/ml in to a roller bottle with a final volume of 300 ml.
3. Incubate the roller bottle at 37°C in a 5 % CO_2/95 % air atmosphere with the vertically orientated bottle shaking at 120 rpm for 3 days until the cells reach a density of $> 2 \times 10^6$ cells/ml.
4. Dilute 300 μg plasmid DNA (total) (*See* Note 2) in 30 ml PBS and vortex briefly.
5. Add 1.2 ml of 0.5 mg/ml PEI to the diluted DNA and vortex briefly.
6. Incubate at room temperature for 20 minutes.
7. Add the PBS, DNA and PEI cocktail to 270 ml cells at 1 x 10^6 cells/ml final concentration.
8. If control of glycosylation is required, add 0.3 ml of 1 mg/ml kifunensine to each roller bottle (*See* Note 4).
9. Incubate the roller bottle at 37°C in a 5 % CO_2/95 % air atmosphere for 48 hrs.
10. To harvest protein: centrifuge cells at 6,000*g* for 5 minutes. For intracellular protein retain cells and store at -80°C and for secreted protein retain supernatant and filter through a 0.22 μm bottle top filter before storing at 4°C.

3.3 Purification of secreted proteins using a His_6 tag

This protocol describes an automated method for purification of secreted proteins from large volumes of media using an Äkta Xpress system (GE Healthcare Life Sciences, UK). However, the initial immobilised metal affinity chromatography (IMAC) purification step (described in section 3.3.2) can be disconnected from the size exclusion chromatography step and automated using other Äkta purification systems.

3.3.1 Automated protocol using the Äkta Xpress

1. Equilibrate either a HiLoad 16/600 Superdex S75 or S200 column with Gel Filtration Buffer (*See* Note 7).
2. Insert buffer lines A1 and A2 into Nickel Wash Buffer and manually wash the pumps to fill the lines with buffer.
3. Insert buffer line A3 into Nickel Elution Buffer. Place a large empty bottle or flask (this needs to be larger than the sample volume) on outlet line F3 and a 96 deep well plate in the fraction collector.
4. Insert a pre-charged 5 ml HisTrap FF column into column position 1 of the Äkta Xpress.
5. Carefully remove line A2 from the Nickel Wash Buffer and insert into the flask containing the filtered protein-containing media.
6. Run the glycoprotein purification programme transcribed in Nettleship *et al.*(27) (*See* Note 8).
7. This programme will complete an automated IMAC purification followed by further purification by size exclusion chromatography giving protein with over 95 % purity (*See* Figure 2).

3.3.2 Description of IMAC purification for use with other systems

1. 50 ml of media is loaded through a 5 ml HisTrap FF column at 8 ml/min followed by 10 ml of Nickel Wash Buffer.
2. Step 1 is then repeated until all the media has been loaded through the column. This load/wash loop reduces the impact of IMAC incompatible components in the media as well as addressing pressure problems due to the viscosity of the mammalian culture medium particularly if it contains FCS.
3. The column is then washed with 50 ml (10 x column volume) of Nickel Wash Buffer before elution of the protein with 25 ml (5 x column volume) Nickel Elution Buffer collecting 2 ml fractions.
4. The product can then be further purified using size exclusion chromatography.

3.4 Purification of intracellular protein using a 3xFLAG® tag

The protocol below describes a method of purification using the 3xFLAG® tag from a 1.2 l transfection. This can be scaled appropriately for larger scale expression. This protocol, including the buffers stated, was developed for the purification of 3xFLAG®-HDAC3:SMRT-DAD complex (*See* Note 9). The method given is manual; however various stages of the process may be automated using Äkta purification systems. After the initial Anti-FLAG® purification, a size exclusion column is used to further purify the protein (including removing the TEV protease).

3.4.1 Initial Anti-FLAG® purification

1. For a 1.2 l scale up, defrost the cell pellet into ~ 30 ml Flag Lysis Buffer.
2. Lyse the cells by sonication using 5 cycles of 30 sec on/30 sec off.

3. Remove the cell debris by centrifugation at 30,000*g* for 30 min at 4°C.
4. Meanwhile, equilibrate 1 ml of packed Anti-FLAG® M2 resin by washing three times with FLAG® Equilibration Buffer.
5. Incubate the supernatant from step 3 with the Anti- FLAG® M2 resin in a 50 ml tube at 4°C for 1 hour.
6. Centrifuge at 1000*g* for 5 min at 4°C. Discard the supernatant and transfer the resin to a 15 ml tube. Wash the resin three times with Flag Wash Buffer 1, then three times with Flag Wash Buffer 2, then three times with Flag Cleavage Buffer.
7. After last wash add 10 ml Flag Cleavage Buffer to the resin along with a1:100 mg\ml dilution of His-TEV protease (*See* Note 10). Incubate overnight at 4°C.
8. Analyse the samples by SDS-PAGE. At this stage this fraction will contain the His-TEV protease as well as the purified protein of interest with the 3xFLAG® tag cleaved. The protein of interest is over 95 % pure discounting the protease (*See* Figures 3 and 4).
9. Before further purification, concentrate the protein to 0.5 ml using an appropriately sized Amicon Ultra Centrifugal filter (*See* Note 11) (Millipore, UK).

3.4.2 Further purification by size exclusion chromatography

1. Based on the molecular weight of the protein of interest and the His-TEV protease, select either a HiLoad Superdex 10/300 S75 or S200 column (*See* Note 7).
2. Equilibrate the size exclusion column in Gel Filtration Buffer.

3. Inject the fractions containing the protein of interest onto the column using a volume lower than 0.5 ml. Larger injection volumes can lead to a loss in resolution.
4. Analyse fractions collected by SDS-PAGE to assess separation of the protein of interest from the TEV protease. The protein of interest is now at sufficient purity (> 99 %) for crystallization and structure determination (23) (*See* Figure 4).

4 Notes

1. Many vectors are available commercially for expression in mammalian cells. In the case of SAP, this was cloned into pOPINTTG which is based on pTT (5) and uses the signal sequence from RTPTμ (28) and adds a C-terminal His_6-tag to the protein. The vector used for the expression of PKMζ and HDAC3 is based on pcDNA3 (Invitrogen, UK) and attaches an N-terminal His_{10}-3xFLAG® -TEV cleavage site tag onto the protein.
2. DNA for transfection needs to have an A_{260}/A_{280} ratio of greater than 1.8. This can be obtained using standard commercial kits such as the PureLink HiPure Plasmid Megaprep kit from Life Technologies, UK.
3. Incubating for longer than 10 minutes can result in loss of transfection efficiency.
4. Kifunensine is an α-mannosidase I inhibitor which results in the secreted product containing only glycans of the form $Man_9GlcNAc_2$ which can be trimmed to one GlcNAc residue using endoglycosidase H (Man = mannose, GlcNAc = N-acetyl glucosamine). This is used to create homogeneous glycans in order to aid crystallogenesis (29).
5. One fully confluent T175 flask of attached cells is used per roller bottle.

6. Suitable roller incubators can be purchased from Wheaton Science products, NJ, USA.
7. The Superdex S75 column resolves proteins in the 3 to 70 kDa molecular weight range and the Superdex S200 column in the 10 to 600 kDa range.
8. The full method for the glycoprotein purification programme is written out in Nettleship *et al.* (27) and can be copied into the Method Editor section of the Unicorn™ software.
9. Depending on the 3xFLAG®-tagged protein being purified, the buffer system can be altered for optimal protein stability. For example, the manufacturer recommends 50 mM Tris-HCl, 150 mM NaCl, pH 7.4 (Sigma Aldrich, UK).
10. Depending on the format of the vector, other proteases such as rhinovirus 3C protease or enterokinase can be used. In addition, the protein may be eluted from the column with its tag intact using the 3xFLAG® peptide (Sigma Aldrich, UK) or a low pH buffer such as glycine-HCl, pH 3.5.
11. When selecting the Amicon Ultra Centrifugal filter to be used, one should select the molecular weight cut off (MWCO) based on half the molecular weight of the protein of interest. This is because the MWCO is calculated using a globular protein model whereas the protein of interest may not be globular.

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Figure legends

Figure 1: Anti-His Western blot showing expression of SAP and PKMζ using HEK 293T cells and *E. coli*. Lanes 1 to 4 show expression of SAP using a signal sequence with lane 1 showing secreted product from HEK cells; lane 2, the HEK whole cell extract; lane 3, secreted product from *E. coli*; and lane 4, *E. coli* whole cell extract. In a similar way, lanes 5 to 8 show expression of SAP without the signal sequence. Lanes 9 to 12 show expression of PKMζ with lane 9 showing whole cell extract from HEKs; lane 10, soluble extract from HEK cells; lane 11, *E. coli* whole cell extract; and lane 12, *E. coli* soluble protein extract. (For information about the vectors used, *see* Note 1).

Figure 2: Example showing the purification of SAP which gave 6 mg from 1 L of media produced via the roller bottle protocol. (a) Size exclusion chromatography trace and (b) SDS-PAGE analysis of the size exclusion fractions.

Figure 3: SDS-PAGE showing the purification of PKMζ from a 30 ml HEK 293F experiment using Anti-FLAG® chromatography. Two constructs of PKMζ were purified with lane 1 showing TEV-cleaved protein from a construct using amino acids 184 to 592 and lane 2, amino acids 231 to 592.

Figure 4: (a) SDS-PAGE showing the purification of HDAC3:SMRT-DAD complex using Anti-FLAG® resin. Lane 1 shows proteins bound to the FLAG® resin. Lane 2 shows the Anti-FLAG® resin after elution of the protein with TEV protease, and Lane 3 shows soluble proteins in the supernatant post elution. (b) Size exclusion chromatography trace (Superdex 200 column) and (c) SDS-PAGE analysis of the size exclusion fractions.

Figure 1

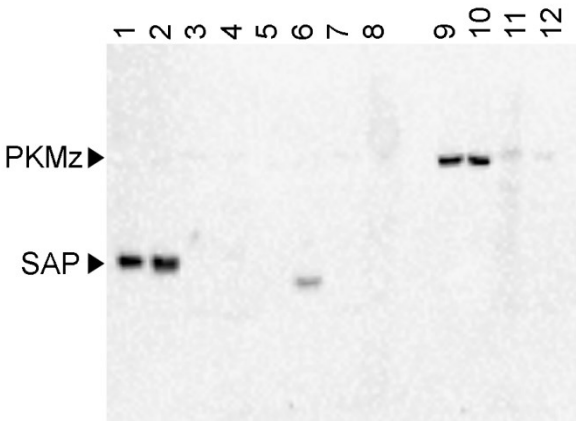


Figure 2A

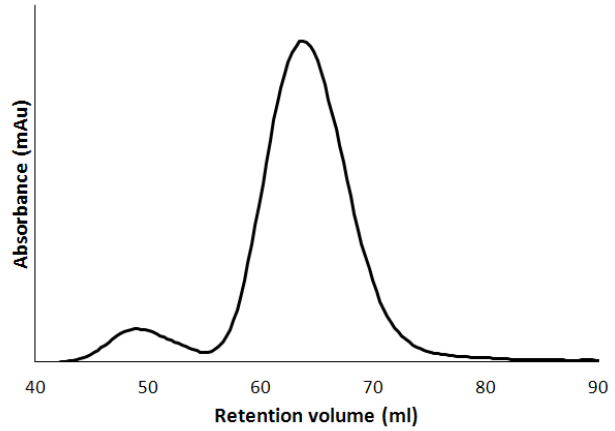


Figure 2B

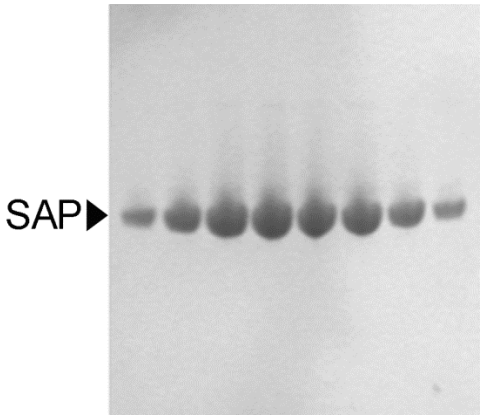


Figure 3

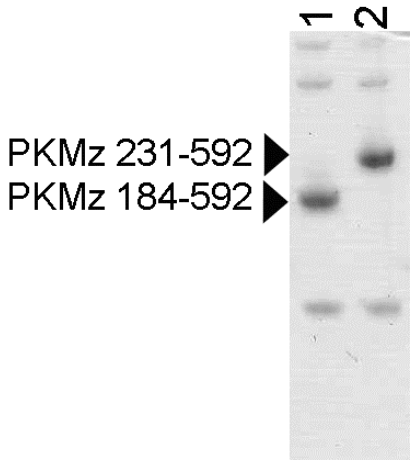


Figure 4A

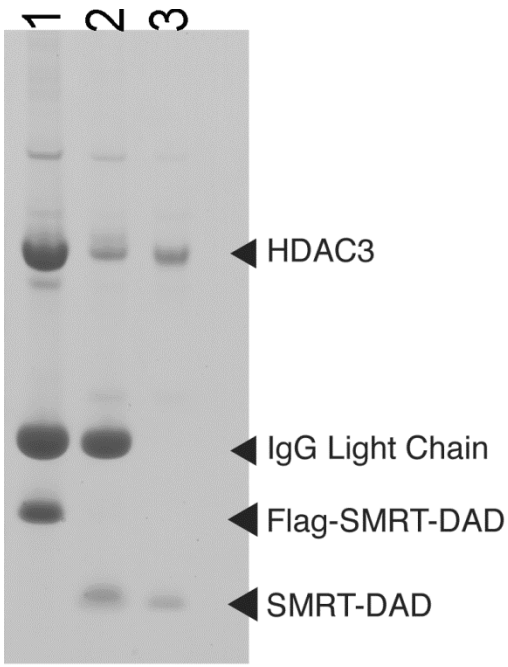


Figure 4B

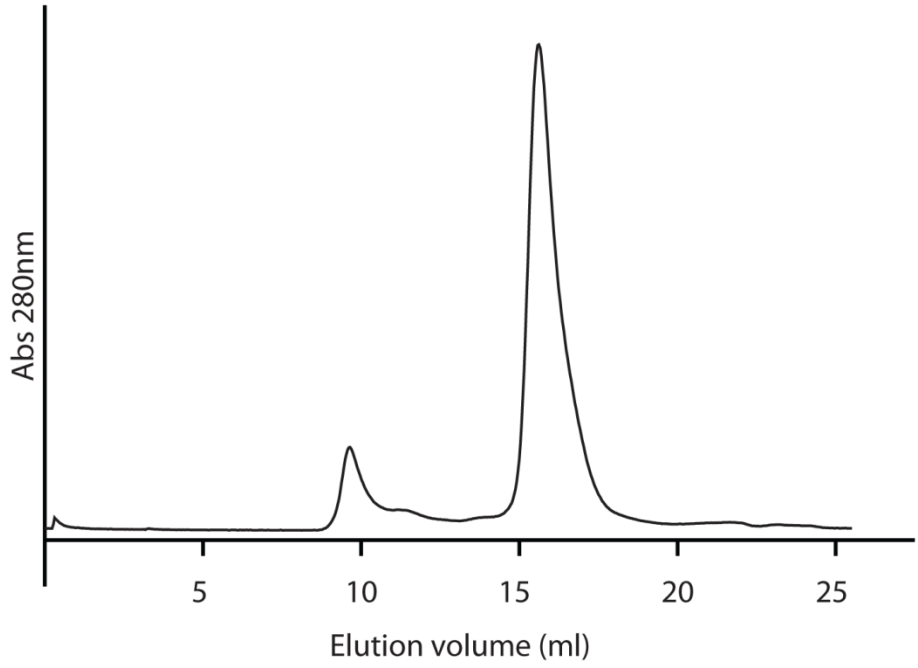
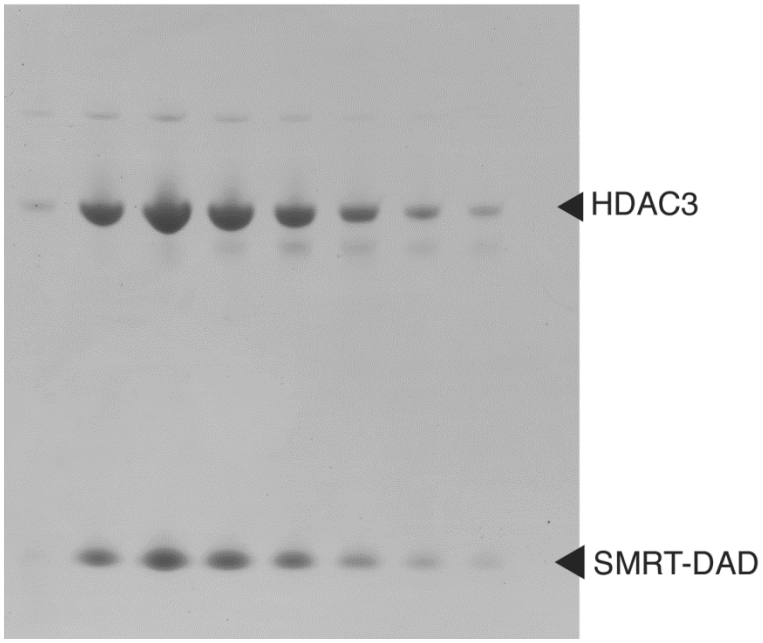


Figure 4C


HDAC3
SMRT-DAD