BL21RIL transformed with DNAJA1 grown in TB with kan/CAM

. 10 mL of overnight used to inoculate 1L TB

. Grow culture to OD600 = ~0.4

. Culture cooled to 16 degC until OD600 = ~0.7-0.8

. induction with 1 mM IPTG overnight

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**Lysis Buffer (adjusted to pH 8)**

Hepes 50 mM

KCl 750 mM

Imidazole 10 mM

bME 6 mM

For every 50 mL lysis buffer, added 1 Roche cOmplete EDTA-free protease inhibitor tablet.

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Clarify lysate by spinning at 30,000xg for 50 minutes.

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**Nickel/High-Salt wash buffer (adjusted to pH 8)**

Hepes 50 mM

KCl 750 mM

Imidazole 10 mM

bME 6 mM

Tween 0.1%

**Nickel/Low-Salt wash buffer (adjusted to pH 8)**

Hepes 50 mM

KCl 200 mM

~~bME 6 mM~~

***\*If do thrombin cleavage, leave out bME starting from this step up to dialysis.\****

**Nickel/Elution buffer (adjusted to pH 8)**

Hepes 30 mM

KCl 200 mM

Imidazole 500 mM

~~bME 6 mM~~

***\*If do thrombin cleavage, leave out bME.\****

**Dialyze out imidazole into buffer (pH adjusted to 8) for 6xHis-tag cleavage:**

Hepes 20 mM

KCl 150 mM

bME 6 mM

**For thrombin cleavage, use this dialysis buffer (pH adjust to 8.4.)**

**Hepes** 20 mM

**KCl** 150 mM

**CaCl2** 2.5 mM

***\*\*\*NO REDUCING AGENT\*\*\****

Once gel analysis indicates cleavage is complete, add bME to buffer & sample to inactivate thrombin for ion-exchange.

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**MonoQ-Buffer A (adjusted to pH 8)**

Hepes 30 mM

KCl 50 mM

bME 6 mM

**MonoQ-Buffer B (adjusted to pH 8)**

Hepes 30 mM

KCl 1 M

bME 6 mM

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**S75 HiLoad 16/60 Buffer (adjusted to pH 7.5)**

Hepes 30 mM

Glycerol 10%

KCl 150 mM

TCEP 1 mM