



# DEOpen User Manual

A guideline for your DEL screening



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WUXI APPTec HITS PLATFORM

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## Part I The DELopen service at WuXi AppTec

### 1. What is DELopen?

WuXi AppTec HitS DNA-Encoded Library (DEL) is a cutting-edge technology that empowers researchers with limitless possibilities in early drug discovery. In our DELopen kit, users are provided with access to more than 4 billion DNA-encoded molecules across series of DEL libraries for the target-to-hit drug discovery campaigns.

DELopen Kit User Manual provided along with the kit will help our researchers to gain better insight and optimized execution of the DEL screening experiments. Detailed description and protocol are included in the User Manual for researchers to review. By reading the User Manual and communicating with WuXi AppTec HitS, researchers would be able to follow the protocol and perform affinity-based DEL selection independently and return the post-selection samples to one of the WuXi AppTec HitS sites around the world for further processing.

A series of processes, including post-selection molecule quantification, sequencing sample preparation, and Next Generation Sequencing (NGS) will be conducted once WuXi AppTec HitS receives the post-selection samples. A summary report (excluding any small molecule structures and DNA sequence information) will be provided to our researchers for further decision on data package unlocking.

The kit includes four vials of the same DELopen libraries (labeled as C1 to C2), DNA-encoded molecules in each vial were additionally labelled with unique DNA-barcode serving as tube identifiers. The strategy is to distinguish molecules in these four vials (C1 to C4) so that they can be used for different selection conditions. These unique identifiers will also enable further PCR amplification to monitor potential cross-contamination during the selection experiment.

## 2. What is in the kit?

Components	Quantity
DEL libraries C1 to C4	4 vials DEL molecules each (10 $\mu$ L/vial)
Post-selection sample collection vials	8 empty vials
Sheared salmon sperm DNA (Cat No. AM9680)	250 $\mu$ L in one tube (10 mg/mL) Final concentration of sssDNA in Selection Buffer is 0.1 mg/mL
Imidazole	1 g (take 0.5 g and dissolve in 7 mL ddH <sub>2</sub> O to make 1 M stock for further usage)
Self-QC package	Buffer A, Buffer B, dipsticks and reaction mix.

For the optimal screening outcomes, the kit should be stored at -80°C and used within 3 months upon arrival. The **Selection Buffer** and **Elution Buffer** are decided and prepared by researchers. Please refer to **Part III. Optimizations and questions (Page 13)** for details of buffer ingredients and other components in DEL screening.

## Part II DEL screening

### 1. Protein QC

#### 1.1 Protein capture test

A prerequisite for a DEL screening is that sufficient amount of the target protein can be immobilized on its corresponding affinity matrix. The entire DEL screening takes place on the immobilized target protein. **Use filter pipette tips all the time.** The protein immobilization test protocol is as follows:

1. Prepare **Selection Buffer** and **Elution Buffer**. Please refer to page 14 for the details of buffer ingredients. sssDNA should be added into **Selection Buffer** to reduce non-specific binding. The final concentration of sssDNA is 0.1 mg/mL in **Selection Buffer**. 10 mM imidazole is required in **Selection buffer** if affinity matrix for polyhistidine-tagged protein is used.
2. Activate the beads. Add 300  $\mu$ L **Selection Buffer** to a fresh 1.5 mL DNA low-binding tube. Pipette 25  $\mu$ L selected beads to the tube and vortex slightly for 5 seconds. Spin down using a mini-centrifuge and place the tube on a magnetic separation rack to separate beads from the buffer. Wait for 15-30 seconds until the solution becomes clear. Remove the supernatant carefully without touching the beads. Add 300  $\mu$ L **Selection Buffer** to repeat the washing step for another two times.
3. Resuspend the beads with 125  $\mu$ L **Selection Buffer** by short vortex, make sure beads are well homogenized. Spin down using a mini-centrifuge. Transfer 25  $\mu$ L of the suspended beads to a fresh 1.5 mL DNA low-binding tube labeled as **Blank Beads**.
4. Place the tube with the remaining beads onto the magnetic separation rack to separate beads from the buffer. Once the solution becomes clear, Remove the supernatant and cap the tube.
5. In a 1.5 mL DNA low-binding tube, dilute 6  $\mu$ g of target protein with **Selection Buffer** to 120  $\mu$ L of volume, gently flick the tube to mix. Spin down using a mini-centrifuge and pipette 20  $\mu$ L of the diluted protein to a fresh 1.5 mL DNA low-binding tube labeled as **Input**.
6. Transfer the rest 100  $\mu$ L of the protein to the beads. Gently flick the tube to disperse the beads in the protein dilution. Incubate the protein with the beads for 30 min at 25°C on a tube rotator/revolver (about 20 rpm). Make sure beads are not clustered on the cap of the tube.
7. After the incubation, spin down the tube using a mini-centrifuge for 5 s and place the tube on the magnetic separation rack. Once the solution becomes clear, use a pipette to carefully

- transfer all the supernatant to a fresh 1.5 mL DNA low-binding tube labeled as **Flow through**.
8. Resuspend the beads with 100  $\mu$ L **Selection Buffer** by **gentle vortex**. Spin down using a mini-centrifuge. Separate beads from the buffer on a magnetic separation rack. Transfer the supernatant to a fresh 1.5 mL DNA low-binding tube labeled as **Wash**.
  9. Resuspend the beads with 100  $\mu$ L **Elution Buffer**, gently flick the tube to make sure beads are well homogenized in the buffer. Transfer 50  $\mu$ L of the beads to a fresh 1.5 mL DNA low-binding tube labeled as **Beads**.
  10. Place the tube with the remaining 50  $\mu$ L beads to a heat block and incubate for 10 min at 95°C with shaking. After incubation, place the heated tube on a magnetic separation rack to separate beads from the supernatant. Carefully transfer the supernatant to a fresh 1.5 mL DNA low-binding tube labeled as **Heated Elution**.
  11. Resuspend heated beads with 50  $\mu$ L **Selection Buffer** and label the tube as **Heated beads**.

## 1.2 SDS-PAGE & Protein Gel Staining

Protein Capture test samples are subject to polyacrylamide gel electrophoresis and protein gel staining to visualize the binding efficiency of the target protein on selected beads. Researchers could choose their own SDS-PAGE system to perform this assay.

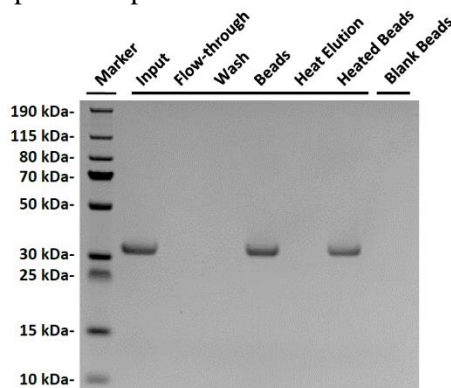
1. Prepare samples for the denaturing gel electrophoresis according to the table below:

	Marker	Input	Flow-through	Wash	Beads	Heated Elution	Heated Beads	Blank Beads
Sample ( $\mu$ L)	5	20	20	20	20	20	20	20
4X Loading Buffer with reducing agent ( $\mu$ L)	-	6.7	6.7	6.7	6.7	6.7	6.7	6.7
Loading volume ( $\mu$ L)	5	15	15	15	15	15	15	15

2. Heat the samples except the marker at 95°C for 5 min in a heat block. Centrifuge the samples at the maximum speed using a benchtop centrifuge.
3. To obtain the best results, it is important to choose the correct gel percentage, buffer system, gel format, and thickness, depending on the specific target protein. In general, designated volume of marker and heated samples are loaded into the wells of a NuPAGE 4 to 12%, Bis-Tris gel for electrophoresis following manufacturers' protocol.

- After electrophoresis is complete, shut off the power, remove the gel from the cassette, and place the gel in a clean staining tray pre-loaded with Brilliant Blue G staining solution (about 20 mL, make sure the gel is immersed). The staining is allowed for 30 min with gentle shaking. The dye is then discarded. The gel is rinsed and immersed in 200 mL deionized water under gentle shaking until a clear background is achieved.
- Use a suitable gel imaging system to visualize the protein capture result.

If the observed pattern on the gel significantly differs from the ideal case displayed here below, **stop** the experimental procedure at this step and contact Technical Support for further guidance at: [DEL\\_service@wuxiapptec.com](mailto:DEL_service@wuxiapptec.com).



### 1.3 Besides protein capture experiment

Besides protein capture test, researchers should also confirm the purity, conformation and activity of the target protein. A 'good' quality protein suitable for DEL screening should be >90% pure, monodisperse, and properly folded. Analytical size-exclusion chromatography can be used to check the monodispersity. A thermal shift assay like nanoDSF is also efficient to check protein folding and validity.

## 2. Affinity selection

### 2.1 Protein immobilization

The protocol described below is designed for one round of a standard DEL screening encompassing 4 conditions.

Prepare **Selection Buffer** and **Elution Buffer**. Please refer to **3 Part III. Optimizations and questions (Page 13)** for the details of buffer ingredients. sssDNA is needed in **Selection Buffer** to reduce non-specific binding, 10 mM imidazole is required in **Selection Buffer** if affinity matrix for polyhistidine-tagged protein is used. **Use filter pipette tips all the time.**

1. Wash the beads by adding 90  $\mu\text{L}$  of homogenized beads to 800  $\mu\text{L}$  **Selection Buffer** in a 1.5 mL DNA low-binding tube. Vortex briefly, spin down, and place the tube on a magnetic separation rack. Carefully pipette out all the supernatant when the separation is complete. Repeat this step for 2 times.
2. After washing, beads are resuspended in 90  $\mu\text{L}$  of **Selection Buffer** and dispensed into four fresh 1.5 mL DNA low-binding tubes labeled as B1 to B4 with 20  $\mu\text{L}$  each. Place B1, B2, B3, and B4 on the magnetic separation rack to separate the supernatant from the beads. Carefully pipette out all the supernatant when the separation is complete.
3. Label 3 fresh 1.5 mL DNA low-binding tubes as P1, P2, and P3. For each tube, dilute 5  $\mu\text{g}$  of corresponding protein with **Selection Buffer** and bring the final volume to 100  $\mu\text{L}$ . Inhibitors/Competitors can be added to the designated tubes in this step.
4. Transfer protein dilutions from P1 to B1, P2 to B2, and P3 to B3. Pipette 100  $\mu\text{L}$  **Selection Buffer** into B4 tube and label it as NTC. Gently flick the tubes to disperse the beads in the solution. Incubate the four tubes at 25°C for 30 min with constant rotation on rotator/revolver (20 rpm). Make sure the beads are not clustered on the cap of the tube. \* *During the incubation time, the DEL solution could be prepared as described in step 1 and 2 from 2.2.1.*
5. Once the incubation is complete, place B1 to B4 tubes on the magnetic separation rack to separate the beads bound with proteins from the supernatant. Carefully remove and discard the supernatant when the separation is complete.
6. Add 200  $\mu\text{L}$  **Selection Buffer** per tube and resuspend the beads by flicking the tubes. Place the tubes on the magnetic separation rack to separate the washing liquid from the beads. Carefully remove the supernatant once the separation finishes and cap the tube immediately. Initiate round 1 selection as soon as possible to avoid beads drying out.



## 2.2 DEL incubation with immobilized proteins

It is essential to decrease the risk of contamination:

- Clean the surface of your lab bench, labware and pipettes with a DNase removal reagent.
- You should use fresh box of filter tips.
- Use new gloves between different rounds of selection rounds.
- Change filter tips between each pipetting step.

### 2.2.1 Round 1 selection

1. Each tube of C1, C2, C3 and C4 provided in the kit contains 10  $\mu\text{L}$  of DEL libraries. Centrifuge the four tubes for 1 min at maximum speed in a benchtop centrifuge.
2. Add 90  $\mu\text{L}$  **Selection Buffer** to each C1 to C4 tubes. Flick the tubes to mix the solutions well. Transfer DEL solutions C1 into the tube containing immobilized protein labeled B1, repeat this step for C2 $\rightarrow$ B2, C3 $\rightarrow$ B3, and C4 $\rightarrow$ B4. Flick the tube to fully mix the DEL with immobilized proteins.
3. Incubate B1-B4 tubes at 25°C for 1 hour with constant rotation on a rotator/revolver (20 rpm). Quick centrifuge the tubes for 3 s to make sure beads are at tube bottom. Place the tubes on the magnetic separation rack to separate supernatant from the immobilized proteins. Carefully pipette out the supernatant completely, avoid touching the beads.
4. Add 200  $\mu\text{L}$  of **Selection Buffer** to B1-B4, gently flick the tubes to fully disperse the beads. Spin down using a mini-centrifuge and put tubes back on the magnetic separation rack to separate post-incubation beads from buffer added. Carefully remove all the buffer and discard it as wash. Repeat this step for 2 times.
5. After washing the beads for 3 times, resuspend the beads with 100  $\mu\text{L}$  **Elution buffer** and incubate the tubes in a heat block at 95°C for 10 min with shaking. After incubation, centrifuge the tube briefly for 5 s.
6. Place the tubes on the magnetic separation rack to separate heated elution from the beads. Carefully transfer all heated elution to the new DNA-low binding tubes labeled as C1-2, C2-2, C3-2, and C4-2.

### 2.2.2 Round N selection (*Selection round N start from 2, maximum is 4*)

- Heated elution collected from Round 1 shall be the DEL input for Round 2 selection.
- Self-QC step is recommended to be performed after Round 2 selection. If a third-round selection is needed, repeat Self-QC after Round 3.
- At least 2 rounds of selection are needed to enrich the potential binders. Performing more

than 4 rounds of selection is not recommended.

- Freshly immobilized proteins should be used in each round of selection.
1. Repeat protein immobilization steps described in **2.1**. Label the tubes accordingly (B1-N, B2-N, B3-N and B4-N). **\*\*\*Number N represents the total selection rounds towards the target protein. N start from 2, maximum is 4.**
  2. Add 1  $\mu\text{L}$  of sssDNA solution into the samples C1-N, C2-N, C3-N, and C4-N obtained from the previous round of selection. Gently flick the tubes to mix the solution well. **\*\*\*Additional imidazole should also be added into the samples to reach the final concentration of 10 mM if affinity matrix for polyhistidine-tagged protein is used.**
  3. Transfer the solution C1-N into the tube containing immobilized protein labeled B1-N and resuspend the beads by flicking the tube gently. Repeat for C2-N $\rightarrow$ B2-N, C3-N $\rightarrow$ B3-N, and C4-N $\rightarrow$ B4-N.
  4. Repeat step 3 to step 5 from **2.2.1**.
  5. Place the tubes on the magnetic separation rack to separate heated elution from the beads. Carefully transfer all heated elution to the new DNA-low binding tubes labeled as C1-(N+1), C2-(N+1), C3-(N+1), and C4-(N+1).

*\* Clients can choose to preserve 50  $\mu\text{L}$  of post-selection samples from the second-last round and send back to WuXi AppTec along with the samples from the last round selection if they feel it is necessary. Compensate the rest of the samples with buffer to 100 $\mu\text{L}$  as input for the next round of selection if there are preservations.*

*\*\*Please label the samples with the sticker of corresponding rounds in the returning box. For example, for the 3<sup>rd</sup> round elution, you should label them as R3-C1, R3-C2, R3-C3, and R3-C4.*

### 3. Self-QC of the post-selection samples

WuXi AppTec HitS strongly recommends researchers to perform real-time, semi-quantification after Round 2 selection to estimate molecule counts of each selection sample. By knowing how many molecules are retained after Round 2 selection, researchers would be able to judge whether a third-round selection is needed.

Researchers could follow the protocol below to practice Self-QC step:

1. Take the QC package out from the freezer 30 min prior to the experiment, thaw **Buffer A** and **Buffer B** at room temperature. Make sure that both buffers are fully thawed. Vortex to make sure each buffer is homogenized. Pre-heat the water bath to 38 °C.
2. Add 46.5 µL **Buffer A** to each reaction tube labeled as **C1**, **C2**, **C3**, **C4**, and **STD** tube, mix well to fully dissolve the lyophilized enzyme and primers mixture. Change pipette tips between tubes to avoid cross contaminations.
3. Add 1 µL of the preserved heated elution from each condition to their corresponding tubes. Add 1 µL of **Standard** to the **STD** tube. (Note: If you receive a tube of **Standard** with lyophilized form. Quick centrifuge the **Standard** tube for 5 min. Add 1 mL ddH<sub>2</sub>O to the tube and vortex to mix well. Then treat the mixed solution as the template sample for the following operation.)
4. Add 2.5 µL **Buffer B** to each reaction tube. For simultaneous reactions across tubes, **Buffer B** can be added in the center of the cap of the tubes and quickly spin down at the same time. Vortex tubes together to mix well.
5. After mixing, quick centrifuge the reaction tubes and put into water bath at 38 °C for 8 min.
6. Swiftly insert reaction tubes into ice to suspend the reaction. Take 10 µL of the reacted solution and dilute in 90 µL ddH<sub>2</sub>O in a 1.5 mL centrifuge tube. Vortex tubes to mix well.
7. Take 5 dipsticks and carefully pipette 60 µL of each dilution made in step 6 on the loading section of the corresponding dipstick. Do not load over the Max line. Read the Target Band intensity 8-10 min after loading the samples and take pictures of the result.

General principles of Real-time semi-quantification:

The intensity of Standard band on Standard dipstick represents 5E8 copies of DEL molecules. T1 band on each condition dipstick represents the actual DEL molecule copy number from post-screening samples.

- If the intensity of the target band is weaker than the Standard band, it indicates the total amount of DEL molecules in one condition is less than 5E8 and the selection should stop at the current round;
- If the intensity of the target band is stronger than or similar to the Standard band, it

indicates the total amount of DEL molecules in one condition is more than 5E8, and another round of selection is needed.

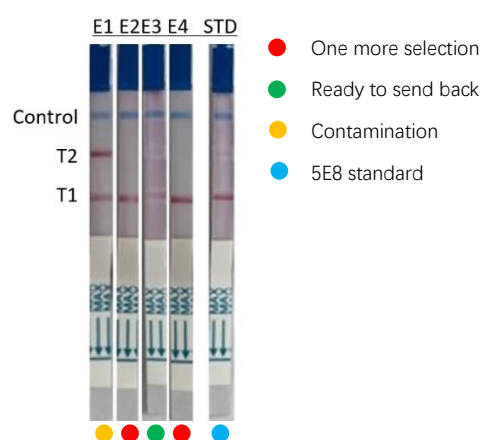
General principle of contamination detection:

The presence of T2 band indicates cross-contamination between conditions. The contamination indicator can help the researcher to examine the operation quality of the screening.

General principle of observation:

The effective observation time is 8 to 10 min post sample loading. All observations are valid under the prerequisite that the Control band on the dipstick appears in each condition. Users could take pictures of the bands during 8 min to 10 min post loading samples to dipsticks

An example of Self-QC is showed here. The blue Control bands at the top indicate the dipsticks are valid and the results are credible. T1 band intensity in STD represents 5E8 molecules. T2 band in E1 represents severe cross-contaminations. T1 bands in E1-E4 indicate the detection of expected DEL molecules. T1 bands in E1, E2, and E4 show similar band intensity as that of STD suggesting another round of selection is needed for E1, E2, and E4. T1 in E3 is weaker than STD suggesting E3 can be stopped at current round and post-selection samples should be sent back to WuXi AppTec HitS for further processing.



There were double-sided tape pre-sticks on the paper holder, and you can tear off the white paper on C1, C2, C3, C4, and STD to stick the corresponding dipsticks on the holder. After all, it will look like the figure below. Please remember to take two pictures at 5 mins and 8-10 mins, your project study director will check your images after receiving your post selection samples:



## 4. Storage and shipping

After the completion of the DEL selection process, you should store all post-selection tubes at -80°C until shipment.

Make sure that dry ice has no impact on tube labels. Fill out the Sample Submission Form with a great care to enable smooth sample processing and decoding.

The shipping address of WuXi AppTec HitS DEL platform is shown below:

Attn: Lin Yuanzhi  
G warehouse #101, No.10 Building,  
#227 Meisheng Road, WaiGaoQiao Free Trade Zone  
Shanghai 200131, China  
Email: lin\_yuanzhi@wuxiapptec.com

- Alternative shipping address for US users:

Attn: Jason Deng  
WuXi AppTec, Suite 111  
22 Strathmore Road Natick  
MA 01760-2434, USA  
Email: Jason.Deng@wuxiapptec.com

- Alternative shipping address for European users:

Attn: Jiajia Zheng  
Crelux GmbH, a WuXi AppTec Company  
Am Klopferspitz 19a  
82152 Martinsried, Germany  
Email: Jiajia.Zheng@wuxiapptec.com

## Part III Optimizations and questions

### 1. The choice of DEL selection buffer

WuXi AppTec HitS DNA-Encoded Library platform encounters many screening cases and we have collected the most commonly used components of our client's screening buffer. The ingredients of **Selection Buffer** and **Elution Buffer** should be adapted from established (published or in-house) functional assay (Biophysics or biochemical) buffer for the target proteins. **Selection Buffer** should contain sssDNA to reduce non-specific binding and if matrix for polyhistidine-tagged protein is used, make sure to include 10 mM imidazole in **Selection buffer** to decrease the non-specific binding. Please use nuclease-free water for buffer preparations.

If clients wish to use small compounds, such as inhibitors or competitors, in **Selection Buffer**, the final concentration of the small compound should be at least 10 times the  $K_d$  or  $IC_{50}$  of this small compound to its target. If there are no  $K_d$  or  $IC_{50}$  data, the final concentration should be at least 10 times the protein concentration.

As for **Elution Buffer**, it should have the same components as **Selection Buffer** except sssDNA, imidazole, small molecules/additives, or any reducing agent like TCEP or DTT.

To initiate selections starting from Round 2, sssDNA should be added into the heated elution from the previous round of screening so that we could convert **Elution Buffer** to **Selection Buffer** for proteins in the further DEL screening. Imidazole and reducing agents should also be added back if needed.

#### Commonly used Selection Buffer:

- 1× PBS buffer containing 0.05% Tween-20, and 0.1 mg/mL sssDNA;
- 1× Tris-HCl buffer containing 0.05% Tween-20, and 0.1 mg/mL sssDNA;
- 1× HEPES buffer containing 0.05% Tween-20, and 0.1 mg/mL sssDNA.

*Please refer to part 2 for detail information.*

## 2. Frequently used reagents in DEL selection?

### For buffering solution:

Name	Useful buffering range	Availability
Tris-HCl	7.5-9.0	Commercial vendor
HEPES	6.8-8.2	Sigma-H3375-100G
PBS	7.2-7.6	Sigma-P5493-1L

Not-listed buffering solution such as MOPS is also acceptable if it suits target protein functional assay. The commonly encountered pH in DEL screening is 6.8 to 9.0. Adjust the pH of imidazole stock to 7.0 during the preparation to avoid pH interfering with the buffer system.

### For salt:

Name	Concentration reference	Availability
NaCl	≤500 mM	Sigma-S5150-1L
MgCl <sub>2</sub>	≤500 mM	Sigma-68475-100ML-F
CaCl <sub>2</sub>	≤500 mM	Sigma-21115-100mL

Higher concentration of salt could decrease the non-specific binding of molecules to the matrix. Over excessive of the salt would potentially deteriorate some proteins and induce static changes in the buffer micro-environment.

### For reducing agent:

Name	Concentration reference	Availability
TCEP	≤5 mM	ST049-5ml
DTT	≤1 mM	B645939-0001

Components such as large amount of amino acid or chemicals with extreme pH are not compatible with DEL screening. Detergents such as 0.05% Tween-20 is recommended in **Selection Buffer** and **Elution Buffer** to decrease the clogging of the beads. Other detergent such as GDN is also acceptable if there is a special need.

### Beads selection

Selection of a proper affinity matrix is important for a DEL screening. Confirmed immobilization of the target protein is one of the key factors for a successful screening. Beads containing large protein or antibody coated surface should be avoided because they bring significant non-specific matrix binding signals. The recommended beads for DEL selections are listed below. Researchers could still use their preferred beads after consulting WuXi AppTec HitS.

Beads	Advantages	Limitations	Solutions
HisPur™ Ni-NTA Magnetic Beads (Thermo-88831)	<p>The most commonly used tag in protein purification.</p> <p>High binding capacity (<math>\geq 40</math> <math>\mu\text{g}</math> green fluorescent protein (GFP)/mg of beads)</p> <p>Low metal ion leaching</p>	<p>Non-specific binding of DEL molecules to HisPur™ Ni-NTA Magnetic Beads.</p> <p>The use of additives that contain chelators such as EDTA or strong reducing reagents such as <math>\beta</math>-mercaptoethanol or DTT could diminish the function of HisPur™ Ni-NTA Magnetic Beads.</p>	<p>Add 10mM imidazole to the Selection Buffer could reduce non-specific binding.</p> <p>TCEP (Tris-(2-carboxyethyl phosphine) can be used as a substitute for DTT.</p>
Pierce™ Streptavidin Magnetic Beads (Thermo 88816)	<p>A commonly used tag for protein purification and it is compatible with biophysics assay like SPR.</p> <p>High binding capacity (~55<math>\mu\text{g}</math> biotinylated rabbit IgG/mg of beads; ~3500<math>\mu\text{mol}</math> biotinylated fluorescein/mg of beads)</p> <p>Low non-specific binding of the proteins</p>	<p>Need to use biotin to block the un-occupied beads after the protein immobilization.</p> <p>Monomeric proteins could be formed into artificial multi-mers on the beads due to the tetrameric structure of the streptavidin on beads.</p>	
Pierce™ Anti-DYKDDDDK Magnetic Beads (Invitrogen-A36797)	<p>High-affinity immobilization of the target protein with the FLAG-tag.</p>	<p>The use of reducing agents such as DTT; <math>\beta</math>-mercaptoethanol or TCEP may denature the Anti-FLAG antibody and thus severely impair the binding capacity of the beads.</p>	<p>Do not use strong reducing agents like DTT, TCEP or <math>\beta</math>-mercaptoethanol. If the protein requires the presence of reducing agent during the selection process, add some Glutathione (GSH) to the buffer.</p>

GST beads is also used by some researchers in protein production and pull-down assays. If the target protein is GST-tagged in DEL screening, researchers should use GST protein itself in the 'NTC' group to remove the GST binders as background noise.



### 3. Q&A

#### 3.1 Protein

3.1.1 Our protein is quite unstable, can we immobilize the protein on the beads at 4 °C instead of 25°C as started in the protocol?

Before you start with affinity selection, you should perform the protein capture test at 4°C. If your protein immobilizes to the beads well at 4°C, you can perform DEL screening at 4°C.

3.1.2 How much protein is needed to do a screening test?

For the protein capture test, the amount of each target protein is 6 µg. For affinity selection, if you do three different targets and one NTC (No target control). 5 µg protein is needed for each condition except for the NTC group in one round of affinity selection.

#### 3.2 DEL screening & Self-QC

3.2.1 What is the timeline of DELopen screening?

It takes about three days to execute the entire experiment. The table below summarizes the average time needed to complete a given step so that you can plan your experiment accordingly. If you are not able to execute all the selection rounds in one day, you may store the buffer and eluent of a selection round at -80 °C before you proceed with the next step

Section	Average Time Required
Protein capture	4 h
Each round of affinity selection	5 h
Post-selection QC	30 min

3.2.2 Based on the Self-QC results, we found that the band in NTC is also detectable and all four samples were homogenous signal intensity. Is it any problem to observe this result?

Based on WuXi experience in DEL screening, the similar molecule quantity shift is common and it does not necessarily suggest that the NTC condition attracted a lot of binders during the

experiment. The binding signal detection can be only finalized after the NGS and data analysis of samples from each condition.

### 3.3 Returning samples:

3.3.1 Regarding the returning sample to WuXi AppTec, will it be problematic if the proteins are included in the returning samples?

It is fine to contain some protein eluents for post-selection samples, we are going to perform the purification once the samples are returned to our molecular platform. Make sure samples are frozen in dry ice for perseverance.

3.3.2 How many dry ice needs to be included in the package to ensure the quality of the post-selection samples in transit?

Generally, 10 kg of dry ice is sufficient for one day of sample transportation, and it is recommended to add another 5 kg of dry ice for each additional day of transportation time. If shipping in summer, please increase the amount of dry ice appropriately.