GenScript DNA-Encoded Chemical Library Kit Guide

[October 2023]

GenScript
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Preparation

User must read the protocol in its entirety before starting the GenDECL screening.

Library Overview

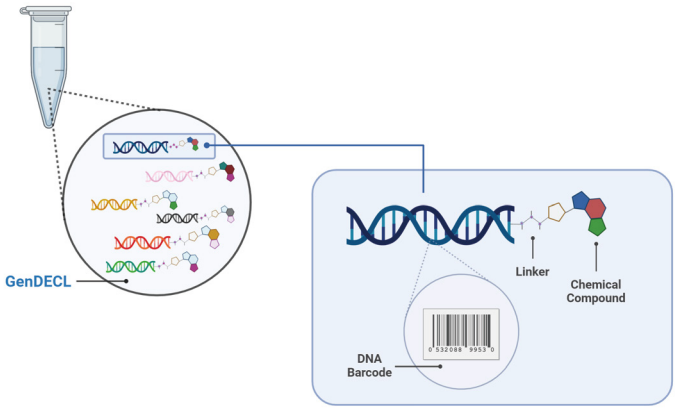


Figure 1. GenScript's DNA‑Encoded Chemical Library (GenDECL). The GenDECL kit consists of a library of diverse drug‑like chemical compounds. Each compound is linked to a unique DNA barcode tag that enables identification of the molecules that may bind with your protein of interest. The library interacts with the target protein, after which the bound compounds are eluted and amplified. Sequencing of the unique barcodes enables identification of compounds that interact with the target protein.

Workflow Overview

Thank you for choosing the GenScript DNA-Encoded Chemical Library (GenDECL) Kit. This manual provides you with comprehensive guidance through the crucial steps and procedures for uncovering the binding properties of your protein of interest. Based on DNA-encoded technology, GenDECL kit is designed to provide you with a convenient, cost-effective, and reliable solution for your research needs while simplifying complex processes such as chemical library development, synthesis, and analysis. Get ready to embark on a journey of enhanced efficiency and scientific discovery with GenDECL!

Protocol Overview

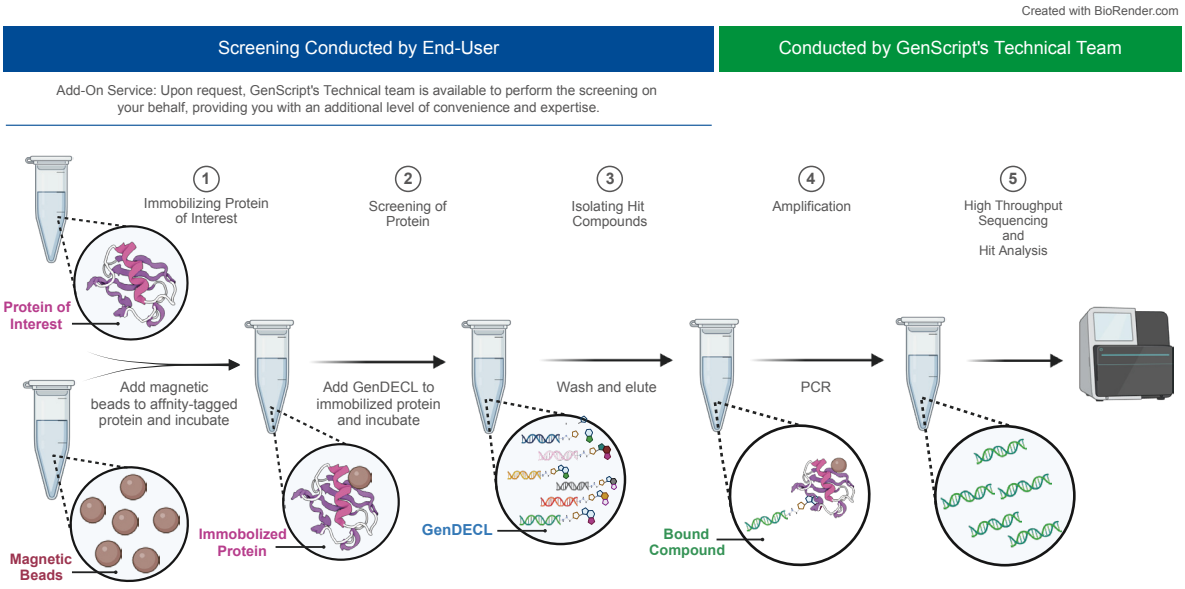


Figure 2. Protocol overview. The following protocol is used to conduct the GenDECL screening against the target protein of interest, using common laboratory equipment. Step 1 through 4 are conducted by the end-user, while step 5 through 7 are conducted by the GenScript technical team.

Add-On service: upon request, GenScript's Technical team is available to perform the screening on your behalf (step 1-4), providing you with an additional level of convenience and expertise.

Step 1. Immobilize the target protein using the appropriate magnetic beads. For example, use Ni-NTA coated magnetic beads for binding and immobilizing of His-tagged proteins.

Step 2. Incubate the protein bound to magnetic beads with the GenDECL library. This incubation step will allow for binding of the library compounds with the target protein.

Step 3. Remove the non-bound compounds by washing.

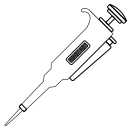
Step 4. Elute the bound compounds off the target protein.

Step 5. The GenScript technical team with amplify the DNA barcodes from the binding compounds using PCR.

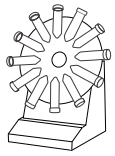
Step 6. The DNA sequence of the PCR product is sequenced.

Step 7. GenScript will analyze the sequencing data, and provide the final analysis report to the Scientist.

Equipment

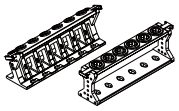


Calibrated hand-held pipette set



Rotating mixer

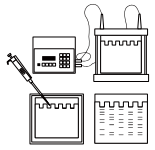
Heating block



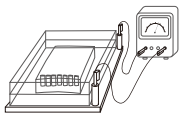
Magnetic rack

AmMag™ MR-mini magnetic rack (Cat. No. L00722) or an equivalent magnetic rack for 1.5 mL centrifuge tubes

Tabletop centrifuge



SDS-PAGE equipment



PAGE equipment or agarose gel electrophoresis equipment

Materials

Product	Recommended Product and Supplier
Magnetic Beads (user can choose Histidine (His), Glutathione S-Transferase (GST), or Flag affinity tags) **Note:** this kit is not designed for Biotinylated or Avidin (AVI) tagged protein screening.	HisPur™ Ni-NTA magnetic Beads Thermo Fisher Catalog Number: 88832
50 mM PBS pH7.4	
Tween-20	
NaCl	
Imidazole	Sigma-Aldrich Imidazole Catalog Number: I5513
Sheared Salmon Sperm DNA	Invitrogen UltraPure™ Herring Sperm DNA Solution Catalog number: 15634017
GenDECL Library Cat. No. L00862	4 vials (5 nmol concentration)
qPCR reagents performed by GenScript	Roche SYBR® Green Catalog Number: 41023820
qPCR forward and reverse primers performed by GenScript	**FP:** 5'-TGACTCCCAAATCGATGTG-3' **RP:** 5'- GCCTCCCTCGCGCCATCAG-3'
PCR reagents for Next Generation Sequencing Library Prep performed by GenScript	GenScript Hot Start PCR Catalog Number: E00049 GenScript dNTP Mix Catalog Number: C01582
PCR Primers performed by GenScript	**FP:** D1R: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG TGACTCCCAAATCGATGTG -3' **RP:** D1F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG GCCTCCCTCGCGCCATCAG -3'

Buffer formulation

Washing Buffer – WB	Selection Buffer – SB
50 mM PBS; pH7.4	50 mM PBS; pH7.4
0.1% Tween-20	0.1% Tween-20
10 mM Imidazole	10 mM Imidazole
150 mM NaCl	150 mM NaCl
	0.1 mg/mL sheared salmon sperm DNA

Protocol

Recommendations

Negative control: with every GenDECL screening, we recommend that non-functionalized beads (beads on which target protein is not bound) are run in parallel as a negative control.

Guidelines for Protein Sample Preparation:

To ensure successful GenScript DECL experiments, please adhere to the following protein sample requirements:

1. His-Tagged Proteins Preferred: While proteins with a His-tag are preferred, it's important to note that proteins with either a His-tag or Flag-tag should not contain EDTA, DTT, or β-mercaptoethanol in the storage buffer.

2. Non-PBS Storage Buffer: If the protein's storage buffer is not PBS and you require GenScript's DECL technical team to conduct subsequent protein screening experiments, kindly provide 100 ml of the storage buffer along with each protein sample.

3. High Protein Purity: We recommend protein purity exceeding 95%. If your sample falls below this threshold, please send a gel image to our GenScript DECL technical team. This will allow us to better assess the suitability of your current protein sample for the experiment.

4. Optimal Protein Concentration: For successful screening, aim for a protein concentration of more than 0.5 μg/μl.

Titrating Your Target Protein:

To optimize the amount of bead-conjugated protein available for interaction with the library, we highly recommend performing a titration of different target protein concentrations during the bead incubation step. This crucial step ensures that you achieve the most efficient and effective results.

As a general guideline, we recommend starting with 5 μg of target protein for each GenDECL screening assay. This starting quantity serves as a general baseline but can be further fine-tuned based on the specific properties of your protein, as

indicated by the band intensity observed on SDS-PAGE after the beads capture test. For instance, if your target protein has a molecular weight exceeding 60 kDa, it is advisable to double the initial recommended amount to 10 μg. By carefully adjusting the protein concentration, you can enhance the precision and reliability of your GenDECL experiments.

Please note that we recommend conducting three rounds of screening. Therefore, if 5 μg of target protein is the required amount for each screening, a total of 15 μg of target protein will be needed for the entire screening process, in addition to the amount used during the bead capture test.

I. Beads capture test

This step confirms successful binding of target protein on the magnetic beads.

1. Take out the magnetic beads from the refrigerator. Shake evenly by inversion or vortexing. Transfer 25 μL of the magnetic beads in a new 1.5 mL microcentrifuge tube, then separate the magnetic beads using the AmMag™ MR-mini magnetic rack, and carefully remove the supernatant without disrupting the bead pallet.

2. Add 200 μL of Washing Buffer (WB) and mix well, then separate the magnetic beads using the AmMag™ MR-mini magnetic rack, and carefully remove the supernatant without disrupting the bead pallet. Repeat the wash step twice, for a total of three washes.

3. Add 125 μL of Selection Buffer (SB) and mix well. Transfer 25 μL of suspended beads to a new 1.5 mL microcentrifuge tube labeled as “Blank Beads”.

4. Separate the remaining magnetic beads using the AmMag™ MR-mini magnetic rack, and carefully remove the supernatant without disrupting the bead pallet.

5. Dilute 6 μg of target protein in 120 μL SB to make the Protein Solution. Transfer 20 μL of the Protein Solution to a new 1.5 mL microcentrifuge tube labeled as “Input”.

Note: we recommend starting with 6 μg of target protein for each GenDECL screening assay. This starting quantity serves as a general baseline but can be further fine-tuned based on the specific properties of your protein, as indicated by the band intensity observed on SDS-PAGE after the beads capture test. For instance, if your target protein has a molecular weight exceeding 60 kDa, it is advisable to double the initial recommended amount to 12 μg.

6. Resuspend the beads from step 4 with the remaining Protein Solution, and incubate for 30 minutes at 25 °C with gentle rocking.

7. Centrifuge the protein-beads complex for a few seconds to collect the reaction. Separate the magnetic beads using the AmMag™ MR-mini magnetic rack, and carefully transfer the supernatant to a new 1.5 mL microcentrifuge tube, labeled as “Flow-through”.

8. Add 200 µL of SB to the protein-beads complex, separate the remaining magnetic beads using the AmMag™ MR-mini magnetic rack, and carefully transfer the supernatant to a new 1.5 mL microcentrifuge tube, labeled as “Wash”.

9. Resuspend the protein-beads complex from step 8 with 100 µL SB buffer. Transfer 50 µL to a new 1.5 mL microcentrifuge tube, labeled as “Beads”. Incubate the remaining 50 µL of the complex at 95℃ for 10 minutes using a heating block.

10. Centrifuge the heated sample for a few seconds. Separate the magnetic beads using the AmMag™ MR-mini magnetic rack, and carefully transfer the supernatant to a new 1.5 mL microcentrifuge tube labeled as “Heated Elution”.

11. Resuspend the beads with 50 µL of SB and label as “Heated Beads”.

12. Prepare SDS-PAGE samples according to the following protocol.

Marker	Input	Flow-through	Wash	Beads	Heated Elution	Heated Beads	Blank Beads
8 µL	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL	10 µL

13. Run SDS-PAGE to confirm successful binding of the target protein on the magnetic beads.

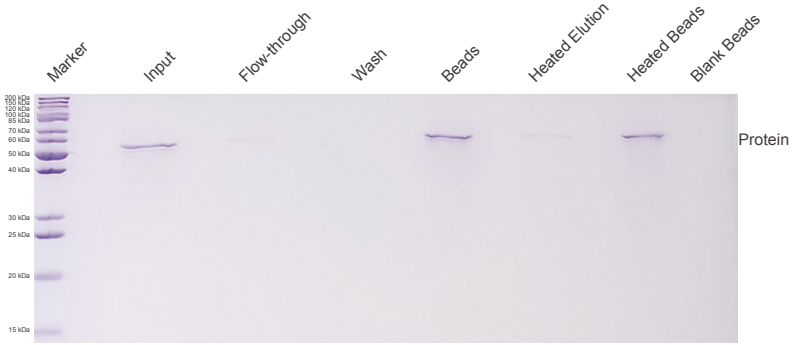


Figure 3. SDS-PAGE Analysis. Sample fractions collected throughout the bead capture test were tested for protein using SDS-PAGE analysis. The “Input” protein sample (protein diluted in SB), The “Beads” sample (protein-beads complex), and the “Heated Beads” sample (magnetic beads from the protein-beads complex after heated elution), all contain the protein of interest, confirming stable biding of the protein of interest on magnetic beads.

Protocol

II. GenDECL Library and Target Binding

Three rounds of screening

Depending on the starting protein amount, a total of 15 - 30 μg protein was used in each experimental group for three rounds of screening. Only 5 nmol GenDECL (one vial) was added in the first round for each sample.

User must save the eluent of the screenings. The eluent after the first screening was used in the second screening. The eluent after the second screening was used for the third screening.

The first round of screening:

1. Take the magnetic beads to be used for protein binding out from the refrigerator, and shake well until the beads are evenly within solution. Transfer 20 μL of beads in a new 1.5 mL microcentrifuge tube for each experimental group, including the negative control, and separate the magnetic beads using the AmMag™ MR-mini magnetic rack, and carefully remove the supernatant without disrupting the bead pallet.

2. Add 200 μL of Washing Buffer (WB) and mix well, then separate the magnetic beads using the AmMag™ MR-mini magnetic rack, and carefully remove the supernatant without disrupting the bead pallet. Repeat the wash step twice, for a total of three washes.

3. Add 100 μL of Selection Buffer (SB) and mix well. Label the 1.5 mL microcentrifuge tube(s), as "**C1-S1**" for sample 1,"**C1-S2**" for sample 2, "**C1-S3**" for sample 3, and "**C1-NTC**" for the negative control.

Note: The second round of screening is labeled as C2-S1; C2-S2; C2-S3; C2-NTC.
The third round of screening is labeled as C3-S1; C3-S2; C3-S3; C3-NTC.

4. Add the required amount of target protein 1, for example 5 μg, into **C1-S1**, target protein 2 into **C1-S2**, and target protein 3 into **C1-S3** accordingly. Incubate for 30 minutes at 25 ℃ with gentle rocking.

Important Note: The recommended 5 μg of target protein is the ideal starting point if 6 μg has been successfully applied in the beads capture tests and has yielded favorable loading results. In cases where 6 μg was not initially used in the capture tests, it is advisable to employ 5/6 of the amount tested during the capture phase. For instance, if the capture test involved 12 μg, then 10 μg would be the appropriate quantity to use. This adjustment ensures optimal compatibility and performance in your GenDECL screening assays.

5. Centrifuge the protein-beads complex for a few seconds to collect the reaction. Separate the magnetic beads using the AmMag™ MR-mini magnetic rack and carefully transfer the clear supernatant to a new 1.5 mL microcentrifuge tube.

6. Add 200 μL of SB to the settled protein-beads complex from step 5, and mix well. Separate the magnetic beads using the AmMag™ MR-mini magnetic rack and carefully remove the supernatant.

7. Dilute each vial of the GenDECL (5nmol), in 100 μL SB. Transfer the 100 μL of the Library Solution to the settled protein-beads complex and negative control from step 6. Mix well, and incubate for 1 hour at 25℃ with gentle rocking.

Note: this incubation step forms the **GenDECL-protein** complex.

8. Centrifuge the complex at full speed for a few seconds to collect the reaction. Separate the magnetic beads using the AmMag™ MR-mini magnetic rack and carefully remove the supernatant.

9. Add 200 μL of SB to the complex and mix well. Separate the magnetic beads using the AmMag™ MR-mini magnetic rack and carefully remove the supernatant without disrupting the bead pallet. Repeat the wash step twice, for a total of three washes.

10. Add 100 µL of SB to the complex and mix well. Incubate at 95 ℃ for 10 minutes to elute.

11. Centrifuge at full speed for a few seconds. Place tubes in the AmMag™ MR-mini magnetic rack.

The second round of screening:

12. Repeat step 1-6 to prepare a fresh protein-beads matrix.

13. Transfer 90 µL supernatant from step 11 to step 12 and add 10 µL SB and mix well. Incubate for 1 hour at 25℃ with gentle rocking.

Note: C1-S1 ⟶ C2-S1 C1-S2 ⟶ C2-S2
C1-S3 ⟶ C2-S3 C1-NTC ⟶ C2-NTC

14. Repeat step 8-11.

NOTE: Following this second round of screening, it is essential to set aside 40 µL of the elution for potential additional qPCR and PCR trials, should there be a limited number of molecules remaining after the third screening step. Consequently, for the third round of screening, we will utilize 50 µL of the elution instead of the previous 90 µL.

The third round of screening:

At this third and final round we have used 15-30 µg of protein in total and reused the 1 vial of 5nmol of GenDECL

15. Repeat step 1-6 to prepare a fresh protein-beads matrix.

16. Transfer 50 µL supernatant from step 14 to step 15 and add 10 µL SB and mix well. Incubate for 1 hour at 25℃ with gentle rocking.

Note: C2-S1 ⟶ C3-S1 C2-S2 ⟶ C3-S2
C2-S3 ⟶ C3-S3 C2-NTC ⟶ C3-NTC

17. Repeat step 8-9.

18. Add 100 µL of ddH_20 to the complex and mix well Incubate at 95 ℃ for 10 minutes to elute. Separate the magnetic beads using the AmMag™ MR-mini magnetic rack and carefully transfer 90 µL of the supernatant to a new 1.5 mL centrifuge tube, labeled as "**1, 2, 3 and NTC**".

Eluted Sample Shipment

Following the elution of your samples, you are kindly requested to send two vials per sample to our GenScript local facilities. Specifically, the first vial should contain half of the elution from the second screening cycle (20 µL), while the second vial should contain the elution from the third screening cycle (50 µL). To ensure the integrity of your samples and to account for any potential shipment issues, we highly recommend retaining half of the elution at your own facilities.

Once the samples are ready for shipment, please contact us at product@gen-script.com to coordinate the correct sample shipment.

We kindly request that you refrain from shipping your samples without the requisite documentation, which will be promptly provided by the GenScript team along with necessary papwer work and comprehensive instructions. Your cooperation in this matter is essential for ensuring the appropriate shipping conditions and accurate delivery to the designated GenScript personnel.

Amplification

performed by GenScript

The eluted samples containing GenDECL library compounds that bound to the target protein are used for PCR-based amplification to generate sufficient signal for sequencing and barcode analysis. However, the number of PCR amplification cycles impact sensitivity of the assay. To identify the optimal number of PCR amplification cycles for your sample, we recommend first performing a qPCR assay as described below.

qPCR Assay Protocol

For best results, plot the cp value to determine the ideal PCR amplification cycles for preparing the sample for NGS.

*The cp value is the PCR cycle number at which your sample's reaction curve intersects the threshold line. This value tells how many cycles it took to detect a real signal from your samples.

Based on the Roche LightCycler® 480 System protocol

Forward Primer	5'-TGACTCCCAAATCGATGTG-3'
Reverse Primer	5'- GCCTCCCTCGCGCCATCAG-3'

Protocol

qPCR Assay Protocol

performed by GenScript

qPCR Reaction Setup	
DNA template	1 µL
Forward Primer (10 µM)	0.5 µL
Reverse Primer (10 µM)	0.5 µL
2x Mix	10 µL
ddH_2O	Up to 20 µL

qPCR Program		
Cycle	**Temperature**	**Time**
1 cycle	95℃	5 minutes
40 cycles	95℃	10 seconds
	60℃	20 seconds
	72℃	10 seconds
1 cycle	95℃	5 seconds
	65℃	1 minutes
	97℃	Continuous
1 cycle	40℃	10 seconds

Sample Preparation for Next Generation Sequencing

performed by GenScript

PCR Protocol

Forward Primer	D1R:5'- GTCTCGTGGGCTCGGAGATGTGTATAA-GAGACAG TGACTCCCAAATCGATGTG -3'
Reverse Primer	5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGA-CAG GCCTCCCTCGCGCCATCAG -3'

Note: the PCR primer regions that are color-coded red are complementarity to the DNA barcode tags of the library.

While the PCR primer regions that are color-coded black, are elective sequences that can be designed to match the NGS sequencing adapters per the sequencing platform.

PCR Reaction Setup	
DNA template	5 µL
D1R (10 µM)	2.5 µL
D1F (10 µM)	2.5 µL
dNTP (2.5 mM)	8 µL
10 x buffer	10 µL
Ex Taq HS	2.5 µL
ddH_2O	Up to 100 µL

PCR Program		
Cycle	**Temperature**	**Time**
Denaturation	98℃	5 minutes
# cycles (determined by qPCR results)	98℃	10 seconds
	68℃	20 seconds
Extension	72℃	10 minutes
Cooling	4℃	HOLD
Cover	105℃	HOLD

Agarose Gel Run

performed by GenScript

After the PCR is complete, run a sample of the product a PAGE gel to check for the size of the amplified fragment. Based on the length of the barcodes, a single band about 150 bp in size is expected.

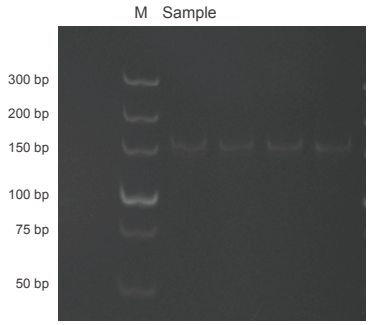


Figure 4. Agarose gel analysis. Upon completion of PCR, samples were analyzed for the DNA size using PAGE gel. As expected, a single band corresponding to the length of the barcodes was observed in all sample lanes.

Final PCR Product Cleanup for Sequencing

performed by GenScript

After the confirmation of the correct band size, proceed to cleaning up your DNA using standard PCR cleanup protocol.

Quantify the DNA concentration in your eluted samples using the NanoDrop or a capillary system. Follow the sequencing platform recommendations to dilute the sample to the appropriate loading capacity for NGS. A single-read sequencing option is recommended.

Protocol

Troubleshooting/Technical Assistance

Contact product@genscript.com

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Make Research Easy

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