Twist High throughput Antibody Production

For Research Use Only (RUO). Not for use in diagnostic procedures.

Twist High Throughput Antibody Production is a gene-to-protein workflow that can produce tens to thousands of diverse antibodies in small-scale quantities for discovery and screening purposes. This document will guide you through the process of preparing your sequences and placing an order within Twist's ordering platform.

The workflow starts with choosing VHH-Fc or conventional IgG antibody expression and submission of the desired antibody sequences into Twist's intuitive ecommerce platform. Bioinformatic screening ensures that potential sequence liabilities that may affect antibody expression are mitigated. Once the order is received, Twist will synthesize and clone antibody genes into a vector of your choosing (either a custom vector or one of Twist's IgG scaffold vectors). Twist antibody engineers will then express, affinity purify, and quality control the requested antibodies.

BEFORE YOU BEGIN

Please ensure you have the following information:

1. **Ensure you are providing trimmed** variable light (VL) and/or variable heavy (VH) amino acid or DNA sequences (it is highly recommended that you review the "How to trim antibody sequences for clonal gene synthesis" section for more information before beginning).

2. Selected vector system:

   a. Twist offers vectors with the following isotype constant regions:

CONVENTIONAL IgG VECTORS		
ISOTYPE	**HEAVY CHAIN VECTOR**	**HEAVY CHAIN CONSTANT REGION**
Human IgG1	pTwist CMV hIgG1*	Human IgG1 constant region
Human IgG2	pTwist CMV hIgG2*	Human IgG2 constant region
Human IgG4	pTwist CMV hIgG4 A228P*	Human IgG4 constant region
ISOTYPE	**LIGHT CHAIN VECTOR**	**LIGHT CHAIN CONSTANT FEATURE**
Human	pTwist CMV hIgGK	κ constant region
Human	pTwist CMV hIgGL2	λ constant region

SINGLE-CHAIN (VHH-FC) VECTORS		
ISOTYPE	**HEAVY CHAIN**	**HEAVY CHAIN FEATURE**
Human IgG1 Fc	pTwist CMV hIgG1-Fc	IgG1 Fc constant region
Human IgG2 Fc	pTwist CMV hIgG2-Fc	IgG2 Fc constant region

EMPTY MAMMALIAN EXPRESSION VECTOR		
ISOTYPE	**VECTOR**	**FEATURE**
Agnostic	pTwist BG CMV WPRE Neo	Available for custom constant region submission

***When co-transfected with the light chain variable region cloned into pTwist hIgGK or hIgGL2, the antibody of interest is produced. Isotype definitions are derived from references 1 and 2.*

b. Please note: **Kozak and Leader peptide (secretion signal) are not included in the Twist Vectors.** These will be added automatically as part of the synthesized insert, unless already included in your submitted sequence.

c. Twist vectors offer the following expression elements within the pTwist CMV vectors:

PTWIST CMV BETAGLOBIN WPRE NEO BASE VECTOR ELEMENTS	
Copy number	High
Promoter	Full-length human cytomegalovirus (CMV) promoter/enhancer for high level gene expression in mammalian cells
WPRE (woodchuck post-transcriptional regulatory element)	Downstream of the cloning site to enhance transcript expression
Resistance	Neomycin resistance gene for selection of stable cell lines
Selection	Mammalian
Intron	Beta globin for enhanced transgene expression
Bacterial Resistance	AmpR encoding for a beta-lactamase gene which confers resistance to ampicillin

CUSTOM VECTOR REQUIREMENTS AND RECOMENDATIONS

If you would like to use a custom vector, please refer to our Custom Vector Onboarding Guide for guidance on onboarding new vectors with Twist Bioscience.

a. Ensure your custom vector is onboarded and available on your eCommerce account. Please contact Customer Support if you do not see your vector.

b. Ensure the following construct requirements are met:
- Promoter
- Kozak sequence—may be appended to variable gene insert
  1. Twist uses the following Kozak sequence: **GCCACC**
- Leader peptide sequence (secretion signal sequence)—may be appended to variable gene insert
  1. Twist uses the following leader sequence for heavy chains: **ATGAAGCATCTGTGGTTCTTTCTGCTCCTGGTTGCCGCTCCCCGGTGGGTTCTGTCC**
  2. Twist uses the following leader sequence for light chains: **ATGGTGCTGCAGACCCAAGTGTTCATCAGTCTGCTGCTTTGGATTTCCGGGGCCTACGGC**
- Trimmed variable Heavy Chain and Light Chain regions
- Constant region sequence
- Stop codons

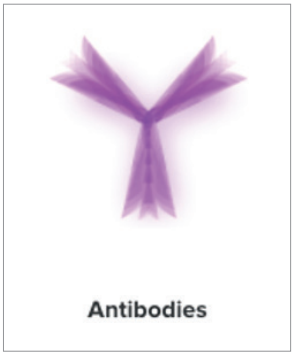
c. Recommended elements to enhance expression:
- Protein expression enhancing introns similar to the beta globin intron present in our pTwist base vector[3,4]
- Major late promoter (MLP) and tripartite leader (TPL) as elements that enhance transcription (MLP) and even mRNA translation due to TPL spliced sequences held in the 5'UTR of an mRNA[5,6]
- Scaffold/matrix attachment region (S/MAR)—elements involved with anchoring DNA to the nuclear matrix to help recruit different factors that aid in DNA replication and gene transcription[7]
- VA RNA I and RNA II—adenoviral non-coding RNAs associated with maintaining productive translation in stressed cells[8]

d. Recommended elements to avoid:
- Redundant elements
- Downstream methionines or stop codons
- Untrimmed sequence elements such as partial constant regions or leader (signal) peptide sequences.

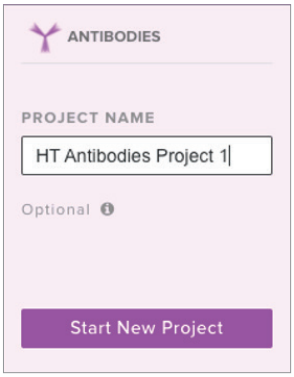
TO GET STARTED

1. Log into your ecommerce account (ecommerce.twistdna.com).

2. Locate the “Antibodies” application on the Home page.



3. Click on the “Antibodies” icon and enter a “Project Name” (optional).



4. Click on “Start New Project” to proceed to the “High Throughput Antibody Production” page.

5. Select the type of antibody you would like to purchase.

- **a.** Select “Single Domain Antibody” for Fc-fusion antibodies, typically a single heavy chain fused to an Fc region. For Single Domain Antibodies, you will need to provide a variable heavy (VH) region sequence.
- **b.** Select “IgG Antibody” for full-length, conventional antibodies. For full-length antibodies, you will need to provide both variable heavy (VH) and variable light (VL) region sequences.
  Note: *If you are submitting antibody sequences with modified constant regions, please select the appropriate antibody type and then select either pTwist CMV BG WPRE Neo or your custom vector as your vector(s) for HC and/or LC.*

6. Read through the “Product Specifications” on the right-hand side of the page to understand the standard offering as well as the options available. Click on “more details” or ⓘ for more information on specific details.

7. Once you are ready to order, select “Order Now”.

Uploading Sequences and Options for Antibody Intake and Clonal Genes

8. To upload your variable region sequences, first select either “Nucleotide Sequence” or “Amino Acid Sequence,” then select your import method.

- **a.** ***Note:*** *If you are selecting pTwist expression vectors, DO NOT include the Kozak and leader sequence while uploading sequences as they will be appended in a subsequent step.*

9. If you are uploading a sequence file for the first time, click on “Download Template”. Open the template to insert the IgG name, VH Name and Sequence (stop here if Single Domain Antibody was selected), VL Name and Sequence (if conventional IgG Antibody was selected). Once complete, select “Upload Sequence File” and either drag and drop your file, or select “Choose Files” and navigate to the file’s location to directly upload.

- **a.** Click “Continue”

10. Select from the following options:

- **a.** IgG Isotype (IgG1, IgG2, IgG4S228P or other if multiple isotypes are being submitted)
- **b.** Light Chain subtype (Lambda or Kappa).
  Please note: *once you select a light chain subtype it will be applied to all the light chain antibodies in your order. if you have multiple light chain types in your order, you will need to select one which will apply that subtype to all light chains and then modify chain types on the following page.*
- **c.** Clonal Gene configuration:
  - **i.** Select vector type for the VH sequence—pTwist expression vector OR an onboarded custom vector
    1. If uploading a variable together with a constant region, please select pTwist CMV WPRE Neo, insertion site NotI-XbaI or a custom vector.
  - **ii.** Select Kozak and leader sequences
    1. If a pTwist expression vector is selected, no modifications are needed in the Kozak and leader sequences
    2. If a custom vector is selected, please use either the “Twist sequence” option or enter the Kozak and leader nucleotide sequences according to the following scenarios:
       - **a.** If both Kozak and leader are fully contained in the custom vector, enter the exact nucleotide sequences contained in the vector
       - **b.** If both Kozak and leader are fully contained in the uploaded insert: enter the exact nucleotide sequences
       - **c.** If the leader is partially contained in the vector: enter the **full** leader nucleotide sequence
         Please note: *when using custom Kozak and leader sequences uploaded in the insert OR contained in the custom vector, an EXACT match to the Kozak and leader nucleotide sequences MUST be entered on the Options page.*
  - **iii.** Select vector type for the VL sequence—pTwist expression vector OR an onboarded custom vector
    1. If uploading a variable together with a constant region, please select pTwist CMV WPRE Neo, insertion site NotI-XbaI or a custom vector.
  - **iv.** Select Kozak and leader sequences
    1. If a pTwist expression vector is selected, no modifications are needed in the Kozak and leader sequences
    2. If a custom vector is selected, please use either the “Twist sequence” option or enter the Kozak and leader nucleotide sequences according to the following scenarios:
       - **a.** If both Kozak and leader are fully contained in the custom vector, enter the exact nucleotide sequences contained in the vector
       - **b.** If both Kozak and leader are fully contained in the uploaded insert, enter the exact nucleotide sequences
       - **c.** If the leader is partially contained in the vector, enter the **full** leader nucleotide sequence

       **Please note* *that upon sequence upload, a bioinformatics screen is run to help identify issues that may impact protein expression. This may take up to several minutes. If you encounter issues, please use the eCommerce chat function at the bottom right of your window or contact customersupport@twistbioscience.com for assistance.*

Review Sequences and Modify Isotypes, Light Chains, or Vector Options

11. Please note that for conventional antibodies, the VH and VL sequences are collapsed under the full-length IgG Name (entered in the sequence upload file).

a. Both the IgG isotype and light chain types can be modified individually or in bulk using the master check box and master drop-down arrow, and the top of the table menu options (highlighted in green circles).

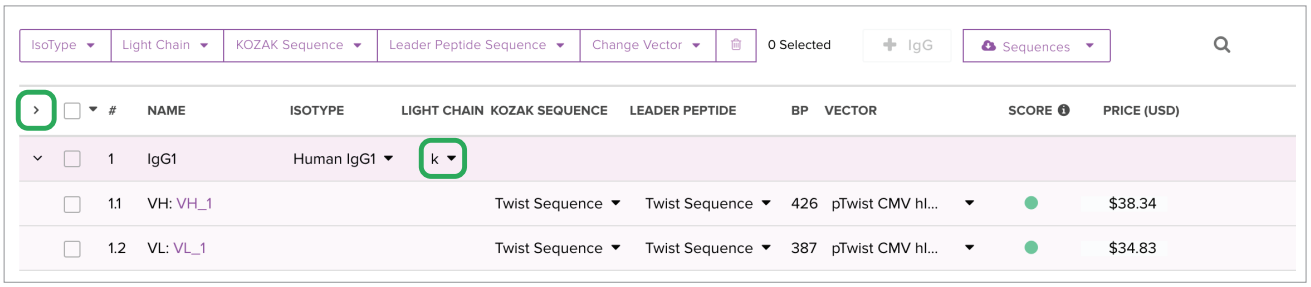
i. To modify light chain type in bulk: select the drop-down arrow next to the master checkbox (located immediately to the left of the “Name” field) select “VL” then hover over the “Light Chain” menu option and select λ or κ.

ii. To modify a single light chain: select the left arrow next to the IgG name to expand the VH and VL line items as pictured. Click the dropdown arrow next to the light chain and select λ or κ.

b. In order to make modifications to the vector or clonal gene settings (Kozak or leader sequence), a similar process may be followed.

i. To modify vectors in bulk: select the dropdown arrow next to the master checkbox (located immediately to the left of the “Name” field) select “VH” or “VL” then hover over the “Change Vector” menu option and select the desired vector.

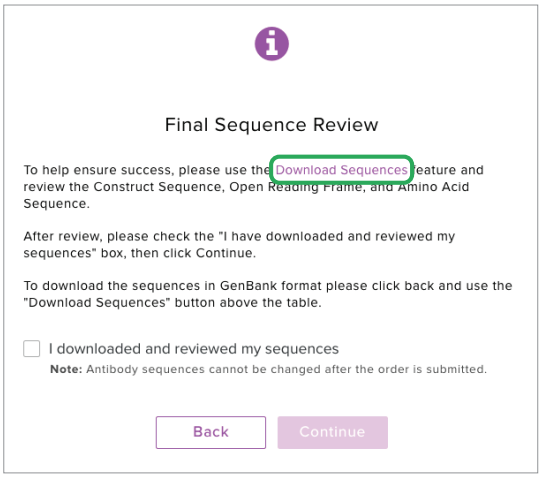
ii. To modify a single vector: click the left arrow next to the name to expand the VH and VL line items as pictured. Click the dropdown arrow next to the Vector and select the desired vector.



**Please note that it will take several seconds to process the bioinformatics screen when making any modifications to the antibody options at this stage.*

iii. Before continuing with your antibody submission please perform a final sequence review. Click on “Download Sequences” and review them carefully.
Note: *sequences cannot be modified once the order is submitted. Click the check box to confirm sequence download and inspection.*

iv. Click “Continue” once your review has been completed. Please pay special attention to each column to ensure the sequence and elements required for successful expression are present.



Select Antibody Production Options

12. Select Production Scale

a. See table for post-purification ranges as well as positive control passing criteria for each production scale.

	1 mL CULTURE	8 mL CULTURE
Typical Post-purification Yield Range	15–300 µg*	15–2,000 µg*
Control Minimum Yield	100 µg	1,000 µg

**Protein yields will vary based on antibody sequence, vector elements, and HC/LC pairing.*

b. Purification scale can ONLY be selected for antibodies submitted with an Fc-region.

i. Submitted antibody sequences must be purifiable with Protein A/G. Twist cannot purify scFv, nanobodies, or Fab fragments. If you are unsure about your antibody's affinity to Protein A/G, please see our FAQs or contact us for additional support.

c. Antibody fragments and antibodies with poor binding affinity to Protein A/G can be produced and sent as supernatants.

13. Select Elution Buffer

a. There are two available Elution buffers to select from:

i. 43 mM Citrate, 148 mM HEPES pH 6 (amine free—recommended for primary amine-based labeling, tethering, or coupling applications)

ii. 83 mM Glycine, 167 mM Tris-HCl pH 6

b. Standard delivery volume is 220 µL for 1 mL scale and 500 µL for 8 mL scale.

14. Select Delivery Format

a. Available formats:

i. 2 mL tube

ii. 96 deep well plate

iii. 1.4 mL Micronic tube

iv. 4 mL Micronic tube

v. 10 mL conical tube (only available for 8 mL supernatant production scale)

Review Shipping, Payment, and Order Details

15. When information is verified as correct and complete, please submit your order.

16. Please note that glycerol stocks are our standard DNA deliverable in addition to the expressed antibodies. If Twist is unable to synthesize and successfully clone an antibody sequence component, you will not receive a glycerol stock or be invoiced for DNA synthesis, cloning and IgG expression and purification for these antibodies.

HOW TO TRIM ANTIBODY SEQUENCES FOR CLONAL GENE SYNTHESIS

A great way to understand if your antibody sequence is properly trimmed for submission is to get familiar with tools for recognizing antibody variable regions. Both VH and VL are composed of V genes and J genes. Within these genes there are conserved segments—frameworks (FWKs) and complementary determining regions (CDRs). FWKs are typically highly conserved and may be used as references for annotations with the help of software such as the Geneious Prime bioinformatics software platform, or bioinformatics tools such as ANARCI.

Below are some available tools to ensure you have isolated and are submitting only the variable regions when either constant region containing pTwist expression vectors are chosen, OR when custom vectors containing Kozak, leader, and constant region sequences are chosen for clonal gene synthesis.

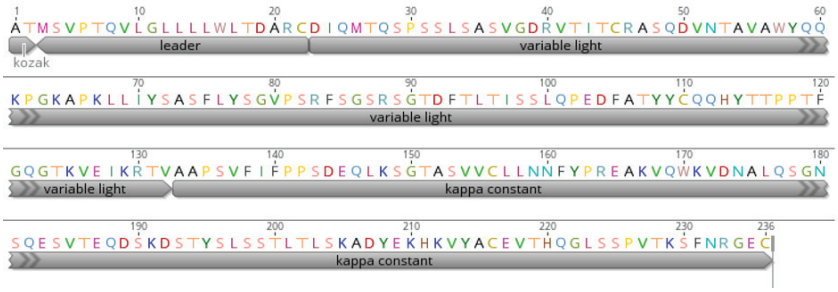
Resources for trimming sequences:

1. abYsis provides an integrated, comprehensive, and annotated sequence assessment for one or multiple antibodies. AbYsis provides a thorough regional breakdown of antibody sequences to quickly identify where your variable region starts and ends. A tabular breakdown of the leader, FWK, and CDR sequences as well as the tail, or constant region sequence is also provided.

   Below is an example readout for the light chain of an example antibody. This readout can be used to annotate sequences which can help guide the submission of the variable regions:

Region	Sequence Fragment	Residues	Length
Leader	ATMSVPTQVLGLLLLWLTDARC	1 - 22	22
LFR1	DIQMTQSPSSLSASVGDRVTITC	23 - 45	23
CDR-L1	RASQDVNTAVA	46 - 56	11
LFR2	WYQQKPGKAPKLLIY	57 - 71	15
CDR-L2	SASFLYS	72 - 78	7
LFR3	GVPSRFSGSRSGTDFTLTISSLQPEDFATYYC	79 - 110	32
CDR-L3	QQHYTTPPT	111 - 119	9
LFR4	FGQGTKVEIKRTV	120 - 132	13
Tail	AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	132 - 236	104
			236

2. It can be helpful to apply the annotations provided by abYsis to your sequence using bioinformatic programs to help visualize and isolate your variable region. Below is an example of a sequence imported to the Geneious Prime software platform with abYsis annotations applied.

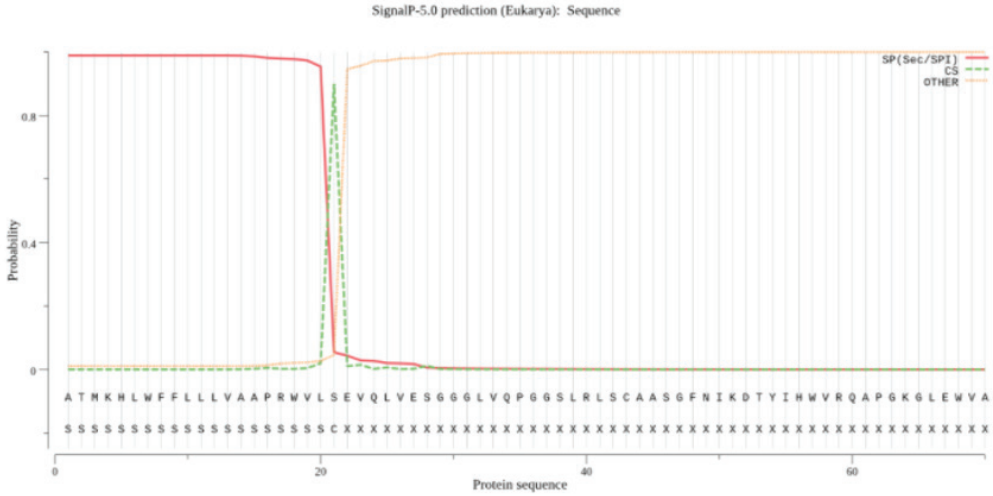


3. A second option for removing leader or signal peptide sequences is SignalP5.0, which is useful for identifying and predicting the presence of signal peptides as well as their cleavage site location in proteins. This may be useful for individuals that have existing leader peptide sequences appended to the variable region, but who would like to have their constructs synthesized in pTwist vectors which require use of the Twist leader sequences.

This program can be used to show cleavage location for several constructs but can take several minutes for processing. Below is an example of identifying the cleavage site for a heavy chain.

Protein type	Signal Peptide (Sec/SPI)	Other
Likelihood	0.9888	0.0112

Download: PNG / EPS / Tabular



4. One additional option that may provide comprehensive breakdown of leader, variable, and constant regions is ANARCI. The resulting analysis provides the exact variable region sequence highlighted in red, which can then be uploaded to the Antibodies application on our eCommerce website. ANARCI also provides detail on the light chain domain type and the sequence species.

5. Lastly, please keep in mind that variable region sequences should never start with a methionine (M). If you are uploading sequences that start with the amino acid M, or nucleotides ATG, and are planning to use pTwist vectors, please contact our support for assistance.

Twist does not assist with configuring, compiling, executing, or troubleshooting the above packages. Twist does not guarantee the output of any of the above packages is accurate, nor does Twist warranty that any referenced package is fit for your particular use.

FAQs

1. How are my antibodies produced?

Twist antibodies are synthesized, cloned, NGS sequence-verified, and produced in a recombinant manner using the HEK293 transient expression system. Antibodies are purified using Protein A/G affinity chromatography. The recombinant production of antibodies increases the likelihood of high yields with batch-to-batch consistency.

2. How are my antibodies inspected for quality?

Twist inspects every antibody product for yield (µg), purity (%), and size (kDa) under reducing conditions. Each antibody will be inspected via LabChip (CD-SDS) to inspect purity and size of the individual heavy and light chains. Yields are quantified using A280 readings. Supernatant samples are quantified using a titer determination method. In the finalized documentation, each antibody will have reported volume, yield, and concentration values in addition to percent purity and heavy chain and light chain sizing. Supernatant documentation will provide antibody titer expression levels of either low, medium or high expression levels.

3. Why was my antibody yield low?

There are a variety of reasons for low antibody yields. It often comes down to either sequence or vector elements that can cause poor expression, poor heavy chain and light chain pairing, poor secretion, or poor affinity for protein A/G. If sequences have any redundancy in leader regions or constant regions, the yields will be much lower than anticipated.

If you are experiencing lower than expected yields, we recommend these troubleshooting steps:

1. First, rule out missing expression elements such as a mammalian Kozak, mammalian leader sequence, or an Fc region compatible with protein A/G purification.

2. Second, validate that the chosen expression vector is compatible with mammalian expression. For example, ensure the vector contains a CMV promoter or other compatible mammalian promoter.

3. Third, it is imperative to review the final constructs provided to you by clicking the “Download Sequence” link. Please check to be sure it **does not** contain the following items:

- Repeated leader sequences
- Partially repeated leader sequences
- Double Signal Sequences
- Partially repeated Fc regions
- Double Fc regions—your insert sequence will be very long!

b. Twist High Throughput Antibody Production expresses your antibody sequences at a fixed volume of cell culture, either the 1 mL or the 8 mL scale. We make every effort to ensure that the expression levels of your IgG are representative of the sequence’s ability to express a protein.

4. Why is my antibody activity different from a prior experiment?

There are a variety of reasons for low or different binding affinities. A few reasons can be attributed to the chosen expression system, elution buffer, quality and purity of the antibody and antigen, or the experimental attributes of the technology used to evaluate affinities. Binding affinities are equilibrium constants. As such, they are subject to changes based on system attributes such as buffer conditions, antibody/antigen purity, and concentrations as well as temperature settings.

Please note: Twist does not screen for antibody affinities and cannot provide QC data relating to binding affinities.

5. What applications can I use my antibodies for and how much antibody is required?

Please see the table below for more information on applications and quantities required:

APPLICATION	MIN. CONCENTRATION	MIN. VOLUME	THROUGHPUT
LSA SPR	0.1 ug/µL (10 µg)	100 µL	High
ELISA	0.15 ug/µL (40 µg)	250 µL	Low
SEC	0.3 µg/µL	60 µL	Low
Epitope Binning using LSA SPR	>0.1 µg (70–100 µg)	>100 µL	High
Thermal Stability	0.5 µg/µL (20 µg)	40 µL	Med
LC-MS	1 mg/mL	1 µL	Low
Endotoxin Testing	0.1 µg/µL	30 µL	Low

6. Can my antibody be purified using Protein A/G affinity chromatography?

Antibodies containing canonical Fc regions can be purified using Protein A/G.

REFERENCES

1. Vidarsson, Gestur et al. “IgG subclasses and allotypes: from structure to effector functions.” Frontiers in immunology vol. 5 520. 20 Oct. 2014, doi:10.3389/fimmu.2014.00520
2. Schroeder, Harry W Jr, and Lisa Cavacini. “Structure and function of immunoglobulins.” The Journal of allergy and clinical immunology vol. 125,2 Suppl 2 (2010): S41-52. doi:10.1016/j.jaci.2009.09.046
3. NOTT, A. “A Quantitative Analysis of Intron Effects on Mammalian Gene Expression.” RNA, vol. 9, no. 5, 1 May 2003, pp. 607–617, 10.1261/rna.5250403.
4. Rose, Alan B. “Introns as Gene Regulators: A Brick on the Accelerator.” Frontiers in Genetics, vol. 9, 7 Feb. 2019, www.frontiersin.org/articles/10.3389/fgene.2018.00672/full, 10.3389/fgene.2018.00672.
5. Berkner, Kathleen L., and Phillip A. Sharp. “Effect of the Tripartite Leader on Synthesis of a Non-Viral Protein in an Adenovirus 5 Recombinant.” Nucleic Acids Research, vol. 13, no. 3, 1985, pp. 841–857, 10.1093/nar/13.3.841.
6. Lu, H et al. “The initiator element of the adenovirus major late promoter has an important role in transcription initiation in vivo.” Journal of virology vol. 71,1 (1997): 102-9. doi:10.1128/JVI.71.1.102-109.1997
7. Narwade, Nitin, et al. “Mapping of Scaffold/Matrix Attachment Regions in Human Genome: A Data Mining Exercise.” Nucleic Acids Research, vol. 47, no. 14, 2 July 2019, pp. 7247–7261, 10.1093/nar/gkz562.
8. Liao, H.-J., et al. “Activities of Adenovirus Virus-Associated RNAs: Purification and Characterization of RNA Binding Proteins.” Proceedings of the National Academy of Sciences, vol. 95, no. 15, 21 July 1998, pp. 8514–8519, 10.1073/pnas.95.15.8514.
9. “Tech Tip #34: Binding characteristics of antibody-binding proteins: Protein A, Protein G, Protein A/G and Protein L” Thermo Fischer Scientific, 2013, TR0034.4, https://tools.thermofisher.com/content/sfs/brochures/TR0034-Ab-binding-proteins.pdf

DOC-001288 REV 3.0