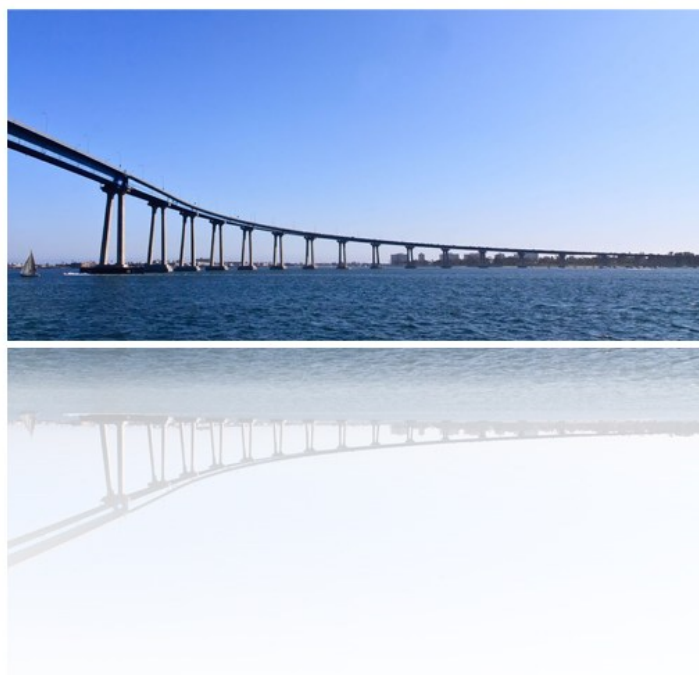




pYDL-12 Yeast Display Vector

INSTRUCTION MANUAL

pYDL-12 Vector for N-terminal Yeast Display with HA tag

Catalog #: YD0100

Version: A1.1 – October 2025

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Description

Introduction

The pYDL-12 vector is a yeast display vector with a cloning site for display on the N-terminal side of the full-length Aga2p protein. Secretion of the fusion protein is driven by the appS4 leader peptide.

The pYDL yeast display vector series offers optimal characteristics for yeast display with tight control of the fusion protein expression, cloning site amenable to multiple cloning strategies and varied display options. The fusion protein is under the control of the *GAL1* promoter, allowing metabolic repression by glucose and induction by galactose. The vector contains two origins of replication, the autonomously replicating sequence along with part of a centromere sequence (CEN/ARS), which allow the vectors to replicate as though they are small single copy independent chromosomes in yeast, and the pMB1 origin of replication derived from pBR322, which results in a high-copy-number plasmid in *E.coli*. The pMB1 sequence lacks the *rop* gene and carries a point mutation in the RNAII transcript G2975 in pBR322 to T1304 on the reverse complement strand responsible for a temperature-sensitive very high copy number phenotype (Lin-Chao 1992).

Content, Shipping & Storage

Content

VECTOR	COMPOSITION	AMOUNT
pYDL-12	20 µl at 0.5 µg/µl of DNA vector in DNA Conservation Buffer (Tris-HCL 5 mM, EDTA 0.1 mM, pH 8.5)	10 µg

Shipping & Storage

pYDL-12 yeast display vector is shipped on wet ice. Upon receipt, store the vector at -20°C.

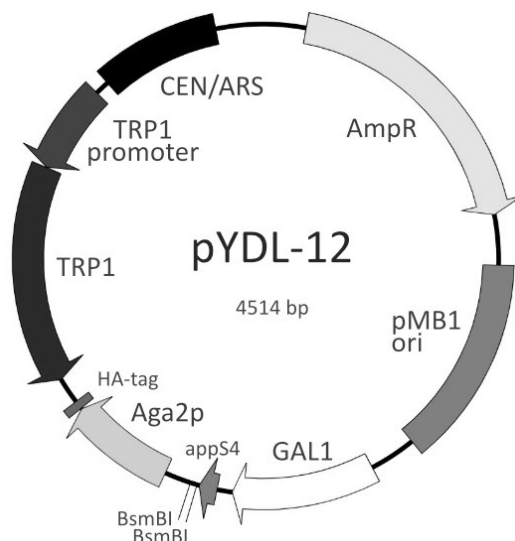
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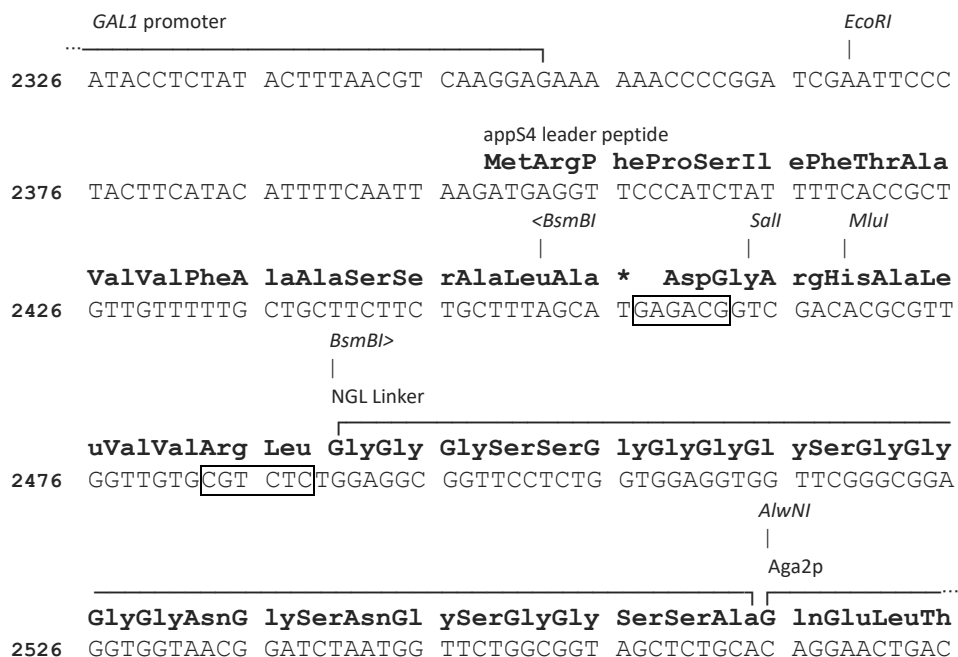
Vector Map

The figure below illustrates the main features of pYDL-12 yeast display vector. The full vector sequence is available online for download in varied formats on the product web page; the total length of the vector is 4514 bp.



Cloning Site

Following is an illustration of pYDL-12 cloning site from the end of the *GAL1* region to the *Aga2p* sequence. The appS4 peptide leader sequence (translation *MRFPSIFTAVVFAASSALA*) is interrupted by a stop codon and the two last amino acids LA will have to be re-introduced during cloning to insure proper reading frame and cleavage (on the C-terminal side of the terminal Alanine).



Feature Table

The features of pYDL-12 yeast display vector are highlighted in the following table.

FEATURE	LOCATION	DESCRIPTION
TEM1 beta-lactamase	132-992	Ampicillin resistance for selection in <i>E. coli</i> .
pMB1 origin	1147-1766	pBR322 origin for replication in <i>E. coli</i> with a high copy-number.
GAL1	1911-2352	GAL1 promoter for galactose-inducible expression of Aga2p fusion protein in <i>S. cerevisiae</i> .
UAS	1911-2028	Upstream activating sequence with several Gal4-binding sites transcriptional activation in the presence of galactose
MIG1 binding sites	2094-2102 2167-2175	Binding sites of the zinc-finger Mig1 to mediate the catabolite repression of <i>Gal1</i> in the presence of glucose.
appS4 leader sequence	2399-2455	Leader peptide sequence for secretion of fusion protein. The missing terminal leucine and alanine will have to be added during the cloning to obtain a complete leader peptide (MRFPSIFTAVVFAASSALA) necessary for proper removal of the leader during the export process.
Aga2p fusion coding sequence	2565-2771	Full-length Aga2p fusion protein coding sequence; the Aga2p protein is fused on it's N-terminal side to the 25 aa linker GGGSSGGGGSGGGNGSNGSGSSA; the exact final sequence of the fusion depends on the cloning strategy (see cloning site).
HA epitope tag	2892-2918	Human influenza hemagglutinin epitope tag coding sequence for C-terminal fusion to Aga2p fusion protein.
TRP1 coding sequence	3673-2999	Coding sequence for phosphoribosylanthranilate isomerase, required for tryptophan biosynthesis and auxotrophic selection in <i>S. cerevisiae</i> .
CEN/ARS	3994-4368	Origin of replication in <i>S. cerevisiae</i> with a stable low copy-number

Restriction Site Summary

Enzyme	Site	Nb	Position	Strand	Isoschizomers
AflIII	A [^] CRYGT	1	2469		
AgeI	A [^] CCGGT	1	1980		AsiGI BshTI CspAI PinAI
AlfI	(10/12) GCANNNNNNTGC (12/10)	1	2950		
ArsI	(8/13) GACNNNNNTTYG (11/6)	1	2636		
BalI	TGG [^] CCA	1	2647		MlsI MluNI Mox20I MscI Msp20I
Bpu10I	CCTNAGC (-5/-2)	1	2851	-	
BssNAI	GTA [^] TAC	1	3704		Bst1107I BstZ17I
Bsu36I	CC [^] TNAGG	1	3427		Eco81I AxyI Bse21I
DrdI	GACNNNN [^] NNGTC	1	1699		AasI DseDI
Eam1105I	GACNNNN [^] NNGTC	1	914		AhdI BmeRI DriI
EcoRI	G [^] AATTC	1	2368		
EcoRV	GAT [^] ATC	1	3386		Eco32I
FalI	(8/13) AAGNNNNNCTT (13/8)	1	3844		
HindIII	A [^] AGCTT	1	3156		
HpaI	GTT [^] AAC	1	3949		KspAI
KpnI	GGTAC [^] C	1	3988		Acc65I Asp718I
MfeI	C [^] AATG	1	3529		MunI
MluI	A [^] CGCGT	1	2469		
MroI	T [^] CCGGA	1	3800		Aor13HI BspEI Bsp13I Kpn2I BseAI
AccIII					
NheI	G [^] CTAGC	1	2925		AsuNHI BmtI BspOI
PsiI	TTA [^] TAA	1	4473		AanI
PsrI	(7/12) GAACNNNNNTAC (12/7)	1	2707	-	

PstI	CTGCA^G	1	2948	BspMAI
PvuI	CGAT^CG	1	547	PleI9I
SapI	GCTCTTC (1/4)	1	4333 -	BspQI LguI PciSI
ScaI	AGT^ACT	1	436	BmcAI ZrmI
SnaBI	TAC^GTA	1	3815	BstSNI Eco105I
SpeI	A^CTAGT	1	2886	AhlI BcuI
Sse8387I	CCTGCA^GG	1	2947	SbfI SdaI
StuI	AGG^CCT	1	2944	Eco147I PceI SseBI
SwaI	ATTT^AAAT	1	4466	SmiI
XbaI	T^CTAGA	1	3585	
XhoI	C^TCGAG	1	4509	Sfr274I PaeR7I SlaI
BauI	CACGAG (-5/-1)	2	250	BssSI Bst2BI
		2	1634	
BseRI	GAGGAG (10/8)	2	1957 -	
		2	2845	
BsgI	GTGCAG (16/14)	2	2560 -	
		2	3277	
BstAPI	GCANNNN^NTGC	2	2226	
		2	3591	
Esp3I	CGTCTC (1/5)	2	2457 -	BsmBI
		2	2483	
NmeAIII	GCCGAG (21/19)	2	791 -	
		2	1924	
PmaCI	CAC^GTG	2	3711	AcvI BbrPI Eco72I PmlI PspCI
		2	4491	

Absent Sites:

AarI, AatII, AbsI, Acc36I, AccB7I, AdeI, AfeI, AjiI, AjuI, AleI, AloI, Aor51HI, ApaI, AscI, AsiSI, AspA2I, AsuII, AvrII, BaeI, BamHI, BarI, BbvCI, BclI, BfuAI, BglI, BglII, BlnI, BlpI, BmgBI, BoxI, BplI, Bpu1102I, Bpu14I, Bsa29I, BsaBI, Bse8I, BseCI, BseJI, BsePI, BseX3I, BshVI, BsiWI, Bsp119I, Bsp120I, Bsp1407I, Bsp1720I, Bsp19I, Bsp68I, BspDI, BspMI, BspT104I, BsrGI, BssHII, BstAUI, BstBI, BstEII, BstENI, BstPAI, BstPI, BstZI, Bsu15I, BsuTUI, BtrI, BtuMI, BveI, CciNI, Cfr42I, Cfr9I, ClaI, CpoI, CsiI, CspCI, CspI, DinI, DraIII, EagI, Ecl136II, EclXI, Eco47III, Eco52I, Eco53kI, Eco91I, EcoICRI, EcoNI, EcoO65I, EcoT22I, EgeI, EheI, FauNDI, FbaI, FseI, FspAI, KasI, KflI, Ksp22I, KspI, MabI, MauBI, Mly113I, Mph1103I, MreI, MroNI, MssI, NaeI, NarI, NcoI, NdeI, NgoMIV, NotI, NruI, NsiI, NspV, OliI, PacI, PaeI, PalAI, PaqCI, PasI, PauI, PciI, PdiI, Pfl123II, PflFI, PflMI, PfoI, PluTI, PmeI, PscI, PshAI, Psp124BI, PspEI, PspLI, PspOMI, PspXI, PsiI, PteI, PvuII, RgaI, RigI, RruI, Rsr2I, RsrII, SacI, SacII, SexAI, SfaAI, SfilI, SfoI, Sfr303I, SfuI, SgfI, SgrAI, SgrBI, SgrDI, SgsI, SmaI, SphI, SrfI, SspDI, SstI, TspMI, Tth111I, Van91I, XagI, XmaI, XmaJI, ZraI, Zsp2I.

Experimental Procedures

General Molecular Biology Techniques

Molecular cloning and yeast display should be conducted under the supervision of a qualified instructor trained to standard safety practice in a molecular biology laboratory environment. Standard molecular biology procedures can be found in a general molecular biology handbook such as Sambrook (1989).

Recommended Yeast Strain

The EBY100 yeast strain is a genetically engineered *Saccharomyces cerevisiae* designed for yeast surface display utilizing the Aga1p-Aga2p a-agglutinin mating complex. The strain has genomic insertion of *AGA1* regulated by the galactose-inducible *GAL1* promoter. This allows for the inducible expression of Aga1p on the cell surface. When a gene for a protein of interest is fused to the Aga2p gene on a pYDL vector and transformed into EBY100, the two subunits (Aga1p and Aga2p-fusion) can be co-expressed and covalently linked by two disulfide bonds, effectively anchoring the protein of interest to the outer surface of the yeast. EBY100 also has multiple auxotrophic markers (e.g., *ura3*, *trp1*, *leu2*) which enable stringent selection using minimal media. Cells can be easily be made competent for transformation by electroporation, suitable for yeast display library generation. The genotype of EBY100 is as follows:

EBY100 *MATa AGA1::GAL1-AGA1::URA3 ura3-52 trp1 leu2-Δ200 his3-Δ200 pep4::HIS3 prbd1.6R can1 GAL*

Plasmid Maintenance

In *E. coli*, propagation and maintenance of pYDL-12 is obtained on any *recA1*, *endA1* strain using LB or 2xYT medium supplemented with ampicillin 100 µg/ml as a selection marker and incubated at 37°C with agitation. The yeast display vector pYDL-12 contains the pBR322 high copy number origin of replication and usually gives high yields of plasmid DNA with most standard laboratory strains such as DH5α or XL10-Gold® from Agilent Technologies, Inc.

In yeast, the pYDL-12 is maintained in tryptophan auxotrophic strains, such as EBY100, using minimal media and incubating at 30°C with agitation. pYDL-12 contains a part of a centromere sequence and autonomously replicating sequence (CEN/ARS) derived from the yeast chromosome, which allows the plasmid to act as an independent chromosome and is typically found as a single copy. Due to the low copy number, we recommend propagating the vector in *E. coli* when larger quantities are required.

Cloning into pYDL-12

In Silico Design

A double inverted BsmBI cloning site immediately follows the appS4 leader peptide sequence and the linker sequence for the Aga2p fusion. After digestion with BsmBI, the CDS to for the protein of interest is inserted in-frame between the two elements in the following illustration. During the cloning, the two BsmBI sites are eliminated.

2435 *AlaAlaSerSe rAlaLeuAla* *GlyGlyG lySerSerG*
 GCTGCTTCTTC TGCTTTAGCA [CDS] GGAGGCG GTTCCTCTG

Vector Digestion

BsmBI alone is sufficient to open the cloning site. Please consult the documentation of your restriction enzyme provider for optimal conditions.

Cloning with FAST-Licase

Homologous recombination is the easiest and most efficient method to clone seamlessly the pYDL-12 vector. We recommend using **FAST-Licase™** (Antibody Design Labs cat# MB101S & MB101L) with the following overhangs. For synthetic dsDNA constructs, the overhangs are added on each side. For PCR-amplified constructs, primers should contain the overhang followed by priming area for the gene of interest. The **FAST-Licase™** reaction contains the insert plus the purified vector digested with BsmBI (see kit instructions).

appS4 overhang 5' – CTGCTTCTCTCTGCTTTAGCA

NGL linker overhang 5' – GGAGGCGGTTCTCTGGTGG (reverse complement for PCR primers)

Primer Design for Restriction Cloning with the BsmBI Sites

Oligo 1 is an example of a primer designed to amplify a CDS sequence and clone it into the BsmBI site situated next to the appS4 leader. A minimum of 2 nucleotides is recommended to cut BsmBI site close to the end (source New England Biolabs); these 2 nucleotides are followed by the BsmBI site CGTCTC and the last five nucleotides of the leader sequence; the resulting NNCGTCTCTAGCA extension is added to the 5' end of the CDS primer.

Oligo1 5' – NNCGTCTCTAGCA–CDS–start

Oligo2 is an example of a primer designed to amplify the CDS from the end including a BsmBI site compatible with pYDL-12 cloning site.

BsmBI is a type IIS restriction enzyme that cuts outside of its recognition site. The second BsmBI site of the cloning site will be cut immediately before the start of the linker sequence for the Aga2p fusion, exactly 1 base after the end of the site and 5 bases further on the opposite strand, thus freeing a 5' 4-base overhang GGAG on the sense strand. In Oligo2, a BsmBI recognition site is situated symmetrically to the vector and will generate a complementary overhang.

Oligo2 5' –NNCGTCTCGCTCC–CDS–end

After digestion and ligation, all the two BsmBI sites, from both insert and vector, will be removed, resulting in a scarless insertion of CDS sequence.

Sequencing of Inserts

The following primers give both strong PCR amplification and sequencing traces.

pydl1a_s 5' – AAGGAGAAAAACCCCGGATC

NGLAga2p_rev 5' – AGTTGTCAGTTCCTGTGCAG

Yeast Transformation

Ensure the correct, in-frame insertion of your gene of interest into the pYDL-12 N-terminal display vector prior to transforming the EBY100 yeast strain. Incorrect cloning will result in failed display. The user should also be familiar with yeast molecular biology and microbiological techniques.

For detailed methodologies on general yeast techniques, refer to *Budding Yeast: A Laboratory Manual* (Andrews et al., 2016). For comprehensive protocols on yeast display applications, consult *Yeast Surface Display: Methods, Protocols, and Applications* (Traxlmayr, 2023). Transform EBY100 with commercial kits or common transformation yeast transformation methods (i.e., lithium acetate). We recommend Frozen-EZ Yeast Transformation II Kit (Zymo Research) to prepare competent yeast for transformation. Competent cells can also be frozen for future use.

Follow the general steps to transform EBY100:

1. Inoculate 5 ml of LB supplemented with carbenicillin with single *E. coli* colonies containing pYDL vector with sequence of interest and incubate at 37°C with shaking overnight.
2. Isolate plasmid DNA for yeast transformation by Miniprep of *E. coli* culture following method of choice.
3. Prepare fresh competent EBY100 yeast following the Zymo Research Frozen-EZ yeast Transformation II Kit protocol or thaw vials of cells from a previously prepared frozen stock on ice.
4. Transform competent cells following the manufacturer's protocol. Incubation time can be increased from 45 min to 2-3 h for increased transformation efficiency.
5. Plate transformation on SDCAA plate (see recipe in appendix) without tryptophan for selection of transformants. Incubate plate at 30°C for 2 to 4 days until single colonies appear.
6. Isolate transformants to test display of fusion proteins after induction.

Induction Conditions & Control of Expression

The expression of the Aga2p fusion protein for yeast display in EBY100 is regulated by the *GAL1* promoter. The *GAL1* promoter is a highly regulated genetic switch in yeast, governing the expression of genes based on available carbon source. This promoter is tightly repressed in the presence of glucose in the medium, and strongly activated by galactose (in the absence of glucose). EBY100 transformants are grown in glucose medium before switching to galactose medium for induction.

The general induction protocol is as follows:

1. Inoculate a single yeast colony into 4 ml of SDCAA containing 2% glucose in a and grow overnight at 30°C with shaking until OD₆₀₀ reaches 6-8.
2. Pellet cells at 2,000 x g for 3 min, and resuspend cells in fresh SDCAA to an OD₆₀₀ of 1. Continue growing the culture at 30°C with shaking until OD₆₀₀ reaches 2-5.
3. Pellet cells as before, aspirate supernatant, and resuspend cells in SGCAA (see recipe in appendix) to an OD₆₀₀ of 1. Induce the culture by incubating at 20°C with shaking overnight.
 - a. **Note:** Growth conditions and type of scaffold affect expression and display on yeast. Induction at 20°C aids the surface expression of less thermally-stable fusions. However, incubation temperature can be raised to 25-37°C to select for stable clones. Induction time can also be increased to up to 72 h with optimal time between 12 – 48 h. We recommend assaying display over 48 h of induction by aliquoting and staining a 1 OD₆₀₀ equivalent number of induced cells at each time point.
4. Induced cells can be analyzed immediately by surface staining or stored at 4°C for several weeks.

Detection of Displayed Proteins

Display of the Aga2p fusion protein on the yeast surface is achieved through antibody staining, utilizing the specific epitope tag encoded by the pYDL vector: HA (for pYDL-12 and pYDL-22) or Myc (for pYDL-13 and pYDL-23). For proper experimental controls, we recommend including uninduced cells as a background control, and untransformed yeast as a negative control for non-specific staining.

To analyze display:

1. Pellet aliquoted yeast in a microcentrifuge tube at 13,000 x *g* for 1 min. Aspirate supernatant and wash with 1 ml of PBS supplemented with 1 mg/ml BSA (PBSA). Repeat wash a second time.
2. Resuspend in 250 µl of PBSA supplemented with 1 µg/ml anti-HA or anti-myc primary antibody.
3. Incubate cells with primary antibody for 0.5 – 1 h at room temperature with periodic mixing or gentle rotation.
4. Pellet cells and wash three times with PBSA as before.
5. Resuspend cells in 250 µl of PBSA supplemented with 2 µg/ml secondary antibody conjugated with fluorescein isothiocyanate (FITC) or any other suitable fluorophore.
6. Incubate cells with secondary antibody for 15-30 min at 4°C with periodic mixing or gentle rotation.
7. Pellet cells and wash three times with PBSA as before.
8. Resuspend cells in 500 µl PBSA for analysis by fluorescent microscopy or flow cytometry.
 - a. **Note:** Keep cells in the dark on ice prior to analysis to minimize photobleaching and remove clumps by filtering cells through cell-strainer capped round-bottom tubes compatible with the flow cytometer.

In optimal conditions, up to 70% of the total cells are easily detectable as displaying the fusion protein by flow cytometry. However, display varies greatly and is highly dependent on the fusion protein and induction conditions. Little to no fluorescence should be observed in uninduced or untransformed yeast.

Appendix

Recipes

Synthetic Dextrose medium with CasAmino Acids (SDCAA), pH 4.5

1. Dissolve the following in 1 L deionized water:
 - a. 20 g dextrose
 - b. 6.7 g yeast nitrogen base
 - c. 5 g casamino acids
 - d. 10.4 g sodium citrate
 - e. 7.4 g citric acid monohydrate
2. Sterilize by filtration
3. Store at 4°C for 1 – 2 months.

SDCAA agar plates, pH 6.0

1. Prepare an agar solution by dissolving the following in 900 ml deionized water:
 - a. 182 g sorbitol
 - b. 15 g agar
 - c. 8.56 g sodium phosphate monobasic monohydrate
 - d. 5.4 g sodium phosphate dibasic anhydrous
2. Autoclave and cool the agar solution to 60°C.
3. Prepare a nutrient mixture by dissolving the following in 100 ml deionized water.
 - a. 20 g dextrose
 - b. 6.7 g yeast nitrogen base
 - c. 5 g casamino acids
4. Sterilize nutrient solution by filtration.
5. Gently mix nutrient solution into the warmed agar solution and pour plates.
6. Store at 4°C for 1 – 2 months.

Synthetic Galactose medium with CasAmino Acids (SGCAA)

1. Dissolve the following in 1 L deionized water:
 - a. 20 g galactose
 - b. 2 g dextrose
 - c. 6.7 g yeast nitrogen base
 - d. 5 g casamino acids
 - e. 8.56 g sodium phosphate monobasic monohydrate
 - f. 5.4 g sodium phosphate dibasic anhydrous
2. Sterilize by filtration
3. Store at 4°C for 1 – 2 months.

MSDS Information

MSDSs (Material Safety Data Sheets) are available on the **Antibody Design Labs** website at the corresponding product page.

Quality Control

Specifications and quality control are detailed on the online product page. **Antibody Design Labs** certifies that the product will perform according to these specifications.

Technical Support

Visit **Antibody Design Labs'** website at **www.abdesignlabs.com** for technical resources, including manuals, vector maps and sequences, application notes, FAQs, etc.

FOR MORE INFORMATION OR TECHNICAL ASSISTANCE, CALL, WRITE, FAX, OR EMAIL US AT:

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