

P09001

Media Starter Package 1



Thank you for purchasing the Media starter package 1 !

Introduction

The Media Starter Package 1 contains all components necessary to prepare the media for carrying out protein interaction screens using the following kits from Dualsystems:

- » DUALmembrane kit (P01001)
- » DUALhunter kit (P01005)
- » DUALhybrid kit (P01004)

The Media Starter Package contains sufficient material for carrying out 5 screens up to and including the stage of library screening and picking of primary positives (Protocol 6 in the DUALmembrane, DUALhunter and DUALhybrid manuals).

For the subsequent analysis of positives from a screen, we recommend the Media Starter Package 2 (P09002), which contains sufficient medium and reagents to analyze 100 prey clones.

Support

Should you encounter any problems during the use of the Media Starter Package, please consult our support pages at www.dualsystems.com. Support protocols and our **Knowledge Base** are constantly updated and hold answers for most commonly encountered problems when working with yeast. If you cannot find answers to your questions in our support pages, contact us at support@dualsystems.com and we will help you as quickly as possible.

Newsletter

We offer a Newsletter with tips & tricks for working with yeast, discussions of recent literature and descriptions of novel products. Please go to www.dualsystems.com to subscribe.

Related products

- » P09002 Media Starter Package 2
- » P06001 Single stranded carrier DNA
- » P06004 Mouse anti LexA antibody
- » P01002 HTX beta-galactosidase assay kit
- » P01003 DS Yeast transformation kit

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1. Kit contents and storage instructions

The Media Starter Package 1 contains the following components:

Component	Supplied as
YPAD media	5 pouches
SD-AHLW media	21 pouches
Adenine	0.2 g powder
L-histidine	0.2 g powder
L-leucine	0.2 g powder
L-tryptophan	0.2 g powder
3-AT	16.8 g powder

Storage and handling of media pouches

Store all media pouches at room temperature. Media are very hygroscopic. Once opened, immediately use all media powder to prepare either liquid or solid media according to the instructions in this manual.

Storage and handling of adenine and amino acids

Store adenine, histidine, leucine and tryptophan powders at room temperature.

Storage and handling of 3-AT

Store 3-AT powder at room temperature.

2. How to use this manual

This manual is divided into three sections:

- » Preparation of stock solutions needed for media preparation
- » Preparation of media for use with the DUALmembrane (P01001) and DUALhunter (P01005) kits
- » Preparation of media for use with the DUALhybrid kit (P01004)

You should start by preparing all stock solutions needed for media preparation. To prepare the actual media for the screen, turn to **Section 4** when working the **DUALmembrane** or **DUALhunter** kit, or to **Section 5** when working with the **DUALhybrid** kit.

3. Preparation of stock solutions

3.1. Required material

- » Adenine, histidine, leucine, tryptophan and 3-AT powders (supplied in the Media Starter Package).
- » 100 ml and 50 ml glass bottles
- » 50 ml Falcon tubes (sterile)
- » 0.2 µm sterile filtration devices
- » Sterile water

3.2. Preparation of adenine, histidine, leucine and tryptophan stock solutions

1. Add the entire contents of each tube to an appropriately sized glass bottle
2. Dissolve contents by adding the amount of water indicated below:

	Supplied amount	Dissolve in
Adenine	200 mg	100 ml
L-histidine	200 mg	20 ml
L-leucine	200 mg	20 ml
L-tryptophan	200 mg	20 ml

3. Shake well until the contents are completely dissolved
4. Sterilize by filtration (we recommend direct filtration into 50 ml Falcon tubes, for example using Steriflip filtration units available from Millipore)
5. Store stock solutions at 4°C

You now have four stock solutions for media preparation. The stock concentrations and the amount of stock solution to add in order to reach the final concentration in the growth medium are given below.

	Stock concentration	Final concentration	Addition to 1 liter of medium
Adenine	2 g/l	10 mg/l	5 ml
L-histidine	10 g/l	20 mg/l	2 ml
L-leucine	10 g/l	100 mg/l	10 ml
L-tryptophan	10 g/l	20 mg/l	2 ml

3.3. Abbreviations for medium components used in this manual

To indicate the composition of defined (SD) media, the following one letter amino acid codes are used:

- » L-tryptophan - W
- » L-leucine - L
- » L-histidine - H

The metabolite adenine is abbreviated as “A”.

3.4. Preparation of 1M aminotriazole (3-AT) stock solution

3-Amino-1,2,4-triazole (3-AT) is a competitive inhibitor of the HIS3 gene product and is commonly used in yeast based screening assays to increase the stringency of selection when using the HIS3 reporter gene.

1. Add the entire contents of the tube into a 250 ml glass bottle
2. Dissolve the contents by adding 200 ml water
3. Add a magnetic stirring bar
4. Stir until completely dissolved (this may take up to an hour since 3-AT has a low solubility in water)
5. Sterilize by filtration (we recommend direct filtration into 50 ml Falcon tubes, for example using Steriflip filtration units available from Millipore)
6. Store the 3-AT stock solution (1M) at -20°C

4. Media preparation for DUALmembrane and DUALhunter kits

4.1. Liquid and agar 1x YPAD Medium

4.1.1. Introduction

Liquid and agar 1x YPAD media are used in Protocols 2 (Transformation of the bait construct into NMY51) and 4 (Verifying expression of your bait construct using the control assay).

4.1.2. Amounts to be prepared for 5 screens

500 ml of liquid 1x YPAD medium and 500 ml of 1x YPAD agar medium

4.1.3. Required material

- » 1 YPAD pouch (supplied in the Media Starter Package)
- » 2 glass bottles (1 liter)
- » 1 magnetic stirring bar
- » 20 sterile plastic petri dishes (10 cm diameter)

4.1.4. Preparation and storage

1. Add the entire contents of 1 YPAD pouch to a 1 liter glass bottle
2. Add a magnetic stirring bar
3. Add 1 liter of water and stir until dissolved completely
4. Transfer 500 ml to a fresh 1 liter bottle and label this "Liquid 1x YPAD"
5. Add 10 g agar to the bottle containing the magnetic stirring bar and label this "1x YPAD agar"
6. Autoclave at 121°C for 15 min
7. Store the liquid 1x YPAD medium at room temperature
8. Place the 1x YPAD agar medium on a magnetic stirrer and stir for 5 minutes to mix all components
9. Label 20 petri dishes with "YPAD" on the side and pour the 1x YPAD agar medium into the dishes
10. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
11. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
12. Store the final agar plates at 4°C

4.2. Liquid 2x YPAD Medium

4.2.1. Introduction

Liquid 2x YPAD medium is used in Protocols 5 (Optimizing the screening stringency using a pilot screen) and 6 (Library transformation and selection of interactors).

4.2.2. Amounts to be prepared for 5 screens

2 liters of liquid 2x YPAD medium

4.2.3. Required material

- » 4 YPAD pouches (supplied in the Media Starter Package)
- » 2 glass bottles (1 liter)

4.2.4. Preparation and storage

1. Add the entire contents of 2 YPAD pouches to each 1 liter glass bottle
2. Add 1 liter of water to each bottle and shake well to dissolve large clumps
3. Autoclave at 121°C for 15 min
4. Store the medium at room temperature

4.3. SD-L, SD-W and SD-LW media

4.3.1. Introduction

In this section, SD-L, SD-W and SD-LW liquid media and agar plates are prepared for use with Protocols 2 to 6.

4.3.2. Amounts to be prepared for 5 screens

Medium	Amount
Liquid SD-L	1300 ml
SD-L agar	300 ml
Liquid SD-W	50 ml
SD-W agar	150 ml
SD-LW agar	1200 ml

4.3.3. Required material

- » 6 pouches of SD-AHLW powder (supplied in the Media Starter Package)
- » Glass beaker (3 liter)
- » 5 glass bottles (1 liter each)
- » 1 glass bottle (500 ml)
- » 2 glass bottles (250 ml each)

- » 7 magnetic stirring bars
- » 21 g Bacto agar
- » 74 sterile plastic petri dishes (10 cm diameter)

4.3.4. Preparation of the SD-LW master medium

1. Empty the entire contents of 6 pouches of SD-AHLW powder into a 3 liter glass beaker
2. Add 3 liters of water
3. Add a magnetic stirring bar and stir until completely dissolved
4. Add 15 ml adenine stock solution
5. Add 6 ml histidine stock solution
6. Aliquot the 3 liters of medium according to the following scheme:
 - » Transfer 650 ml of SD-LW master medium to each of two 1 liter bottles and continue with Section 4.3.5
 - » Transfer 300 ml of SD-LW master medium to a 1 liter bottle and continue with Section 4.3.6
 - » Transfer 50 ml of SD-LW master medium to a 250 ml bottle and continue with Section 4.3.7
 - » Transfer 150 ml of SD-LW master medium to a 250 ml bottle and continue with Section 4.3.8
 - » Transfer 600 ml of SD-LW master medium to each of two 1 liter bottles and continue with Section 4.3.9

4.3.5. Preparation of liquid SD-L medium

1. Add 1.3 ml tryptophan stock solution to each bottle
2. Autoclave the medium at 121 °C for 15 min
3. Store the autoclaved medium at room temperature

4.3.6. Preparation of SD-L agar medium

1. Add 0.6 ml tryptophan stock solution to the bottle
2. Add 6 g Bacto agar
3. Add a magnetic stirring bar
4. Autoclave the medium at 121 °C for 15 min
5. Place the agar medium in a 50 °C water bath for 1-2 hours
6. Place the agar medium on a magnetic stirrer and stir for 5 minutes
7. In the meantime, prepare 13 petri dishes (10 cm diameter) by marking with "SD-L" on the side
8. Pour the agar medium into the petri dishes
9. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
10. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
11. Store the final agar plates at 4 °C

4.3.7. Preparation of liquid SD-W medium

1. Add 0.5 ml leucine stock solution to the bottle
2. Autoclave the medium at 121 °C for 15 min
3. Store at room temperature

4.3.8. Preparation of SD-W agar medium

1. Add 1.5 ml leucine stock solution to the bottle
2. Add 3 g Bacto agar
3. Add a magnetic stirring bar
4. Autoclave the medium at 121 °C for 15 min
5. Place the agar medium in a 50 °C water bath for 1-2 hours
6. Place the agar medium on a magnetic stirrer and stir for 5 minutes
7. In the meantime, prepare 7 petri dishes (10 cm diameter) by marking with “SD-W” on the side
8. Pour the agar medium into the petri dishes
9. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
10. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
11. Store the agar plates at 4 °C

4.3.9. Preparation of SD-LW agar medium

1. Add 12 g Bacto agar to each of the bottles
2. Add a magnetic stirring bar
3. Autoclave the medium at 121 °C for 15 min
4. Place the agar medium in a 50 °C water bath for 1-2 hours
5. Place the agar medium on a magnetic stirrer and stir for 5 minutes
6. In the meantime, prepare 54 petri dishes (10 cm diameter) by marking with “SD-LW” on the side
7. Pour the agar medium into the petri dishes
8. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
9. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
10. Store the agar plates at 4 °C

4.4. SD-HLW agar medium

4.4.1. Introduction

In this section, SD-HLW agar plates with increasing amounts of 3-AT are prepared for use with Protocols 4 (Verifying expression of your bait using the control assay) and 5 (Optimizing the screening stringency using a pilot screen).

4.4.2. Amounts to be prepared for 5 screens

Medium	Amount
SD-HLW agar medium	2500 ml

4.4.3. Required material

- » 5 pouches of SD-AHLW powder (supplied in the Media Starter Package)
- » 3-AT stock solution (supplied in the Media Starter Package)
- » 3 liter glass beaker
- » 7 glass bottles (1 liter)
- » 8 magnetic stirring bars

- » 49.6 g Bacto agar
- » 22 petri dishes (10 cm diameter)
- » 30 petri dishes (15 cm diameter)

4.4.4. Preparation of SD-HLW agar medium

1. Add the entire contents of 5 pouches of SD-AHLW powder to the glass beaker
2. Dissolve the contents by adding 2.5 liters of water
3. Add 12.5 ml adenine stock solution
4. Add a magnetic stirring bar and stir until the contents are dissolved completely
5. Label seven 1 liter glass bottles with "A" to "G".

Note

Be sure to use a label which is not removed during autoclaving. We recommend writing onto the autoclave indicator tape with a waterproof pen

6. Aliquot the SD-HLW medium into the bottles and add the appropriate amount of Bacto agar according to the scheme below:

	Amount of SD-HLW medium to add	Amount of Bacto agar to add
Bottle A	500 ml	10 g
Bottle B	330 ml	6.6 g
Bottle C	330 ml	6.6 g
Bottle D	330 ml	6.6 g
Bottle E	330 ml	6.6 g
Bottle F	330 ml	6.6 g
Bottle G	330 ml	6.6 g

7. Add a magnetic stirring bar to each bottle
8. Autoclave the medium at 121 °C for 15 min
9. Place the agar medium in a 50 °C water bath for 1-2 hours
10. Add the appropriate amount of 3-AT stock solution to each bottle according to the table below:

	Volume of 3-AT stock solution [1M]	3-AT final concentration
Bottle A	-	-
Bottle B	-	-
Bottle C	0.330 ml	1.0 mM
Bottle D	0.875 ml	2.5 mM
Bottle E	1.650 ml	5.0 mM
Bottle F	2.475 ml	7.5 mM
Bottle G	3.300 ml	10.0 mM

11. Place each bottle on a magnetic stirrer and stir for 5-10 minutes to ensure proper mixing of all components
12. In the meantime, prepare the petri dishes according to the table below:

	Number and type of petri dish	Label
Bottle A	22 petri dishes (10 cm diameter)	SD-HLW
Bottle B	5 petri dishes (15 cm diameter)	SD-HLW
Bottle C	5 petri dishes (15 cm diameter)	SD-HLW / 1 mM 3-AT
Bottle D	5 petri dishes (15 cm diameter)	SD-HLW / 2.5 mM 3-AT
Bottle E	5 petri dishes (15 cm diameter)	SD-HLW / 5 mM 3-AT
Bottle F	5 petri dishes (15 cm diameter)	SD-HLW / 7.5 mM 3-AT
Bottle G	5 petri dishes (15 cm diameter)	SD-HLW / 10 mM 3-AT

13. Pour the medium of each bottle into the appropriate type and number of petri dishes, as indicated in the Table in Step 12
14. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
15. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
16. Store the agar plates at 4°C

4.5. SD-AHLW agar medium

4.5.1. Introduction

In this section, SD-AHLW agar plates with increasing amounts of 3-AT are prepared for use with Protocols 4 (Verifying expression of your bait using the control assay) and 5 (Optimizing the screening stringency using a pilot screen).

4.5.2. Amounts to be prepared for 5 screens

Medium	Amount
SD-AHLW agar medium	2500 ml

4.5.3. Required material

- » 5 pouches of SD-AHLW powder (supplied in the Media Starter Package)
- » 3-AT stock solution (supplied in the Media Starter Package)
- » 3 liter glass beaker
- » 7 glass bottles (1 liter)
- » 8 magnetic stirring bars
- » 49.6 g Bacto agar
- » 22 petri dishes (10 cm diameter)
- » 30 petri dishes (15 cm diameter)

4.5.4. Preparation of SD-AHLW agar medium

1. Add the entire contents of 5 pouches of SD-AHLW powder to the glass beaker
2. Dissolve the contents by adding 2.5 liters of water
3. Add a magnetic stirring bar and stir until the contents are dissolved completely
4. Label seven 1 liter glass bottles with "A" to "G".

Note

Be sure to use a label which is not removed during autoclaving. We recommend writing onto the autoclave indicator tape with a waterproof pen

5. Aliquot the SD-AHLW medium into the bottles and add the appropriate amount of Bacto agar according to the scheme below:

	Amount of SD-HLW medium to add	Amount of Bacto agar to add
Bottle A	500 ml	10 g
Bottle B	330 ml	6.6 g
Bottle C	330 ml	6.6 g
Bottle D	330 ml	6.6 g
Bottle E	330 ml	6.6 g
Bottle F	330 ml	6.6 g
Bottle G	330 ml	6.6 g

6. Add a magnetic stirring bar to each bottle
7. Autoclave the medium at 121 °C for 15 min
8. Place the agar medium in a 50 °C water bath for 1-2 hours
9. Add the appropriate amount of 3-AT stock solution to each bottle according to the table below:

	Volume of 3-AT stock solution [1M]	3-AT final concentration
Bottle A	-	-
Bottle B	-	-
Bottle C	0.330 ml	1.0 mM
Bottle D	0.875 ml	2.5 mM
Bottle E	1.650 ml	5.0 mM
Bottle F	2.475 ml	7.5 mM
Bottle G	3.300 ml	10.0 mM

10. Place each bottle on a magnetic stirrer and stir for 5-10 minutes to ensure proper mixing of all components
11. In the meantime, prepare the petri dishes according to the table below:

	Number and type of petri dish	Label
Bottle A	22 petri dishes (10 cm diameter)	SD-AHLW
Bottle B	5 petri dishes (15 cm diameter)	SD-AHLW
Bottle C	5 petri dishes (15 cm diameter)	SD-AHLW / 1 mM 3-AT
Bottle D	5 petri dishes (15 cm diameter)	SD-AHLW / 2.5 mM 3-AT
Bottle E	5 petri dishes (15 cm diameter)	SD-AHLW / 5 mM 3-AT
Bottle F	5 petri dishes (15 cm diameter)	SD-AHLW / 7.5 mM 3-AT
Bottle G	5 petri dishes (15 cm diameter)	SD-AHLW / 10 mM 3-AT

12. Pour the medium of each bottle into the appropriate type and number of petri dishes, as indicated in the Table in Step 11

13. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
14. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
15. Store the agar plates at 4 °C

4.6. Preparation of selective medium for the library screen

4.6.1. Introduction

In this section, the selective plates required for the library screen in the DUALmembrane or DUALhunter system are prepared. The type of selective medium to prepare depends on the outcome of the pilot screen carried out according to Protocol 5 of the DUALmembrane or DUALhunter manuals.

4.6.2. Amounts to be prepared for 1 screen

Please note that the amounts indicated below are for one screen only, as it is unlikely that all baits you have prepared for screening will require the same type of selective plates. Therefore, you should prepare selective plates for the library screen only after you have determined the proper selection conditions in Protocol 5.

Selective medium	Amounts required for 1 screen
As determined in Protocol 5	16 petri dishes (15 cm diameter) 1 liter selective medium

4.6.3. Required material

- » 1 pouch of SD-AHLW powder (supplied in the Media Starter Package)
- » 3-AT (supplied in the Media Starter Package)
- » 1 glass bottle (1 liter)
- » Magnetic stirring bar
- » 16 petri dishes (15 cm diameter)

4.6.4. Preparation of selective medium for one screen

1. Add the contents of the entire SD-AHLW pouch to the glass bottle
2. Dissolve the contents in 1 liter water
3. **If your selective medium is SD-HLW (with or without 3-AT),** add 5 ml adenine stock solution. **If your selective medium is SD-AHLW (with or without 3-AT),** omit this step
4. Add 20 g Bacto agar
5. Add a magnetic stirring bar
6. Autoclave the medium at 121 °C for 15 min
7. Place the medium in a 50 °C water bath for 1-2 hours
8. **If your selective medium contains 3-AT,** add the appropriate amount of 3-AT stock solution, as indicated in the Table below. **If your selective medium does not contain 3-AT,** omit this step

3-AT final concentration	Volume of 3-AT stock solution [1M] to add
1.0 mM	1.0 ml
2.5 mM	2.5 ml
5.0 mM	5.0 ml
7.5 mM	7.5 ml
10.0 mM	10.0 ml

9. Place the bottle on a magnetic stirrer and stir for 5-10 minutes to ensure proper mixing of all components
10. In the meantime, label 16 petri dishes (15 cm diameter) with the selection conditions
11. Pour the medium into the petri dishes
12. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
13. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
14. Store the agar plates at 4°C

To prepare the selective media for analyzing your positive clones from the screen, we recommend the Media Starter Package 2 (P09002).

5. Media preparation for the DUALhybrid kit

5.1. Liquid and agar 1x YPAD Medium

5.1.1. Introduction

Liquid and agar 1x YPAD media are used in Protocol 2 (Testing your bait for self-activation).

5.1.2. Amounts to be prepared for 5 screens

500 ml of liquid 1x YPAD medium and 500 ml of 1x YPAD agar plates

5.1.3. Required material

- » 1 YPAD pouch (supplied in the Media Starter Package)
- » 2 glass bottles (1 liter)
- » 1 magnetic stirring bar
- » 20 sterile petri dishes (10 cm diameter)

5.1.4. Preparation and storage

1. Add the entire contents of 1 YPAD pouch to a 1 liter glass bottle
2. Add a magnetic stirring bar
3. Add 1 liter of water and stir until dissolved completely
4. Transfer 500 ml to a fresh 1 liter bottle and label this "Liquid 1x YPAD"
5. Add 10 g agar to the bottle containing the magnetic stirring bar and label this "1x YPAD agar"
6. Autoclave at 121°C for 15 min
7. Store the liquid 1x YPAD medium at room temperature
8. Place the 1x YPAD agar medium on a magnetic stirrer and stir for 5 minutes to mix all components
9. Label 20 petri dishes with "YPAD" on the side and pour the 1x YPAD agar medium into the dishes
10. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
11. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
12. Store the final agar plates at 4°C

5.2. Liquid 2x YPAD Medium

5.2.1. Introduction

Liquid 2x YPAD medium is used in Protocols 4 (Optimizing the screening stringency using a pilot screen) and 6 (Library transformation and screening for interactors).

5.2.2. Amounts to be prepared for 5 screens

2 liters of liquid 2x YPAD medium

5.2.3. Required material

- » 4 YPAD pouches (supplied in the Media Starter Package)
- » 2 glass bottles (1 liter)

5.2.4. Preparation and storage

1. Dissolve 4 pouches “YPAD” in 2 liter water
 2. Prepare 4 bottles with 500 ml medium each
 3. Autoclave the medium at 121 °C, 15 min
 4. Store at room temperature
1. Add the entire contents of 2 YPAD pouches to each 1 liter glass bottle
 2. Add 1 liter of water to each bottle and shake well to dissolve large clumps
 3. Autoclave at 121 °C for 15 min
 4. Store the medium at room temperature

5.3. SD-W and SD-LW media

5.3.1. Introduction

In this section, SD-W and SD-LW liquid media and agar plates are prepared for use with Protocols 2 to 6.

5.3.2. Amounts to be prepared for 5 screens

Medium	Amount
Liquid SD-W	1300 ml
SD-W agar	300 ml
SD-LW agar	900 ml

5.3.3. Required material

- » 5 pouches of SD-AHLW powder (supplied in the Media Starter Package)
- » Glass beaker (3 liter)
- » 3 glass bottles (1 liter each)
- » 1 glass bottle (500 ml)
- » 5 magnetic stirring bars
- » 24 g Bacto agar
- » 53 sterile plastic petri dishes (10 cm diameter)

5.3.4. Preparation of the SD-LW master medium

1. Empty the entire contents of 5 pouches of SD-AHLW powder into a 3 liter glass beaker
2. Add 2.5 liters of water

3. Add a magnetic stirring bar and stir until completely dissolved
4. Add 12.5 ml adenine stock solution
5. Add 5 ml histidine stock solution
6. Aliquot the 2.5 liters of medium according to the following scheme:
 - » Transfer 650 ml of SD-LW master medium to each of two 1 liter bottles and continue with Section 5.3.5
 - » Transfer 300 ml of SD-LW master medium to a 500 ml bottle and continue with Section 5.3.6
 - » Transfer 900 ml of SD-LW master medium to a 1 liter bottle and continue with Section 5.3.7

5.3.5. Preparation of liquid SD-W medium

1. Add 6.5 ml leucine stock solution to each bottle
2. Autoclave the medium at 121 °C for 15 min
3. Store the autoclaved medium at room temperature

5.3.6. Preparation of SD-W agar medium

1. Add 3 ml leucine stock solution to the bottle
2. Add 6 g Bacto agar
3. Add a magnetic stirring bar
4. Autoclave the medium at 121 °C for 15 min
5. Place the agar medium in a 50 °C water bath for 1-2 hours
6. Place the agar medium on a magnetic stirrer and stir for 5 minutes
7. In the meantime, prepare 13 petri dishes (10 cm diameter) by marking with “SD-W” on the side
8. Pour the agar medium into the petri dishes
9. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
10. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
11. Store the final agar plates at 4 °C

5.3.7. Preparation of SD-LW agar medium

1. Add 18 g Bacto agar to the bottle
2. Add a magnetic stirring bar
3. Autoclave the medium at 121 °C for 15 min
4. Place the agar medium in a 50 °C water bath for 1-2 hours
5. Place the agar medium on a magnetic stirrer and stir for 5 minutes
6. In the meantime, prepare 40 petri dishes (10 cm diameter) by marking with “SD-LW” on the side
7. Pour the agar medium into the petri dishes
8. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
9. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
10. Store the agar plates at 4 °C

5.4. SD-HLW agar medium

5.4.1. Introduction

In this section, SD-HLW agar plates with increasing amounts of 3-AT are prepared for use with Protocol 4 (Optimizing the screening stringency using a pilot screen).

5.4.2. Amounts to be prepared for 5 screens

Medium	Amount
SD-HLW agar medium	1500 ml

5.4.3. Required material

- » 3 pouches of SD-AHLW powder (supplied in the media kit)
- » 3-AT stock solution (supplied in the media kit)
- » 3 liter glass beaker
- » 4 glass bottles (500 ml)
- » 5 magnetic stirring bars
- » 30 g Bacto agar
- » 24 petri dishes (15 cm diameter)

5.4.4. Preparation of SD-HLW agar medium

1. Add the entire contents of 3 pouches of SD-AHLW powder to the glass beaker
1. Dissolve the contents by adding 1.5 liters of water
2. Add 7.5 ml adenine stock solution
3. Add a magnetic stirring bar and stir until the contents are dissolved completely
4. Label four 500 ml glass bottles with "A" to "D".

Note

Be sure to use a label which is not removed during autoclaving. We recommend writing onto the autoclave indicator tape with a waterproof pen

5. Aliquot the SD-HLW medium into the bottles and add the appropriate amount of Bacto agar according to the scheme below:

	Amount of SD-HLW medium to add	Amount of Bacto agar to add
Bottle A	375 ml	7.5 g
Bottle B	375 ml	7.5 g
Bottle C	375 ml	7.5 g
Bottle D	375 ml	7.5 g

6. Add a magnetic stirring bar to each bottle
7. Autoclave the medium at 121 °C for 15 min
8. Place the agar medium in a 50 °C water bath for 1-2 hours
9. Add the appropriate amount of 3-AT stock solution to each bottle according to the table below:

	Volume of 3-AT stock solution [1M]	3-AT final concentration
Bottle A	-	-
Bottle B	0.375 ml	1.0 mM
Bottle C	0.937 ml	2.5 mM
Bottle D	1.875 ml	5.0 mM

10. Place each bottle on a magnetic stirrer and stir for 5-10 minutes to ensure proper mixing of all components
11. In the meantime, prepare the petri dishes according to the table below:

	Number and type of petri dish	Label
Bottle A	6 petri dishes (15 cm diameter)	SD-HLW
Bottle B	6 petri dishes (15 cm diameter)	SD-HLW / 1 mM 3-AT
Bottle C	6 petri dishes (15 cm diameter)	SD-HLW / 2.5 mM 3-AT
Bottle D	6 petri dishes (15 cm diameter)	SD-HLW / 5 mM 3-AT

12. Pour the medium of each bottle into the appropriate type and number of petri dishes, as indicated in the Table in Step 11
13. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
14. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
15. Store the agar plates at 4 °C

5.5. SD-AHLW agar medium

5.5.1. Introduction

In this section, SD-AHLW agar plates with increasing amounts of 3-AT are prepared for use with Protocol 4 (Optimizing the screening stringency using a pilot screen).

5.5.2. Amounts to be prepared for 5 screens

Medium	Amount
SD-AHLW agar medium	1500 ml

5.5.3. Required material

- » 3 pouches of SD-AHLW powder (supplied in the media kit)
- » 3-AT stock solution (supplied in the media kit)
- » 3 liter glass beaker
- » 4 glass bottles (500 ml)
- » 5 magnetic stirring bars
- » 30 g Bacto agar
- » 24 petri dishes (15 cm diameter)

5.5.4. Preparation of SD-AHLW agar medium

1. Add the entire contents of 3 pouches of SD-AHLW powder to the glass beaker
1. Dissolve the contents by adding 1.5 liters of water
2. Add a magnetic stirring bar and stir until the contents are dissolved completely
3. Label four 500 ml glass bottles with "A" to "D".

Note

Be sure to use a label which is not removed during autoclaving. We recommend writing onto the autoclave indicator tape with a waterproof pen

4. Aliquot the SD-AHLW medium into the bottles and add the appropriate amount of Bacto agar according to the scheme below:

	Amount of SD-AHLW medium to add	Amount of Bacto agar to add
Bottle A	375 ml	7.5 g
Bottle B	375 ml	7.5 g
Bottle C	375 ml	7.5 g
Bottle D	375 ml	7.5 g

5. Add a magnetic stirring bar to each bottle
6. Autoclave the medium at 121 °C for 15 min
7. Place the agar medium in a 50 °C water bath for 1-2 hours
8. Add the appropriate amount of 3-AT stock solution to each bottle according to the table below:

	Volume of 3-AT stock solution [1M]	3-AT final concentration
Bottle A	-	-
Bottle B	0.375 ml	1.0 mM
Bottle C	0.937 ml	2.5 mM
Bottle D	1.875 ml	5.0 mM

9. Place each bottle on a magnetic stirrer and stir for 5-10 minutes to ensure proper mixing of all components
10. In the meantime, prepare the petri dishes according to the table below:

	Number and type of petri dish	Label
Bottle A	6 petri dishes (15 cm diameter)	SD-AHLW
Bottle B	6 petri dishes (15 cm diameter)	SD-AHLW / 1 mM 3-AT
Bottle C	6 petri dishes (15 cm diameter)	SD-AHLW / 2.5 mM 3-AT
Bottle D	6 petri dishes (15 cm diameter)	SD-AHLW / 5 mM 3-AT

11. Pour the medium of each bottle into the appropriate type and number of petri dishes, as indicated in the Table in Step 10
12. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
13. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
14. Store the agar plates at 4 °C

5.6. Preparation of selective medium for the library screen

5.6.1. Introduction

In this section, the selective plates required for the library screen in the DUALhybrid system are prepared. The type of selective medium to prepare depends on the outcome of the pilot screen carried out according to Protocol 4 of the DUALhybrid manual.

5.6.2. Amounts to be prepared for 1 screen

Please note that the amounts indicated below are for one screen only, as it is unlikely that all baits you have prepared for screening will require the same type of selective plates. Therefore, you should prepare selective plates for the library screen only after you have determined the proper selection conditions in Protocol 4.

Selective medium	Amounts required for 1 screen
As determined in Protocol 4	16 petri dishes (15 cm diameter) 1 liter selective medium

5.6.3. Required material

- » 1 pouch of SD-AHLW powder (supplied in the media kit)
- » 3-AT (supplied in the media kit)
- » 1 glass bottle (1 liter)
- » Magnetic stirring bar
- » 16 petri dishes (15 cm diameter)

5.6.4. Preparation of selective medium for one screen

1. Add the contents of the entire SD-AHLW pouch to the glass bottle
2. Dissolve the contents in 1 liter water
3. **If your selective medium is SD-HLW (with or without 3-AT),** add 5 ml adenine stock solution. **If your selective medium is SD-AHLW (with or without 3-AT),** omit this step
4. Add 20 g Bacto agar
5. Add a magnetic stirring bar
6. Autoclave the medium at 121 °C for 15 min
7. Place the medium in a 50 °C water bath for 1-2 hours
8. **If your selective medium contains 3-AT,** add the appropriate amount of 3-AT stock solution, as indicated in the Table below. **If your selective medium does not contain 3-AT,** omit this step

3-AT final concentration	Volume of 3-AT stock solution [1M] to add
1.0 mM	1.0 ml
2.5 mM	2.5 ml
5.0 mM	5.0 ml
7.5 mM	7.5 ml
10.0 mM	10.0 ml

9. Place the bottle on a magnetic stirrer and stir for 5-10 minutes to ensure proper mixing of all components

10. In the meantime, label 16 petri dishes (15 cm diameter) with the selection conditions
11. Pour the medium into the petri dishes
12. Wait for the agar to become solid (1-2 hours), invert the agar plates and incubate at room temperature overnight to remove excess liquid
13. Place the agar plates in a plastic pouch or wrap with parafilm to avoid drying out
14. Store the agar plates at 4°C

To prepare the selective media for analyzing your positive clones from the screen, we recommend the Media Starter Package 2 (P09002).

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Contact information

Dualsystems Biotech AG
Grabenstrasse 11a
8952 Schlieren-Zurich
Switzerland

Phone +41 44 738 5000
Fax +41 44 738 5005