

P09002

Media Starter Package 2



Thank you for purchasing the Media starter package 2 !

Introduction

The Media Starter Package 2 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 analyzing up to 100 positive yeast clones from a DUALmembrane, DUALhunter or DUALhybrid screen (Protocols 8 and 9 in the DUALmembrane and DUALhunter manuals, Protocols 7 and 8 in the DUALhybrid manual).

To prepare media for the initial steps of a screen (bait testing and library screening) we recommend the Media Starter Package 1 (P09001).

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

- » P09001 Media Starter Package 1
- » 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

The Media Starter Package 2 contains the following components:

Component	Supplied as
YPAD media	1 pouch
SD-AHLW media	11 pouches
Adenine	0.2 g powder
L-histidine	0.2 g powder
L-leucine	0.2 g powder
L-tryptophan	0.2 g powder
Aminotriazole (3-AT)	4.2 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 and tryptophan 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 100 ml glass bottle
2. Dissolve the contents by adding 50 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 1x YPAD Medium

4.1.1. Introduction

Liquid 1x YPAD medium is used in Protocol 9 (Confirmation of positive interactors).

4.1.2. Amounts to be prepared for analysis of 100 preys

1 liter of liquid 1x YPAD medium

4.1.3. Required material

- » 1 YPAD pouch (supplied in the Media Starter Package)
- » 1 glass bottle (1 liter)

4.1.4. Preparation and storage

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

4.2. SD-L and SD-LW media

4.2.1. Introduction

In this section, SD-L and SD-LW liquid media and agar plates are prepared for use with Protocols 8 (Plasmid recovery from yeast and retransformation into *E. coli*) and 9 (Confirmation of positive interactors).

4.2.2. Amounts to be prepared for the analysis of 100 preys

Medium	Amount
Liquid SD-L	250 ml
Liquid SD-LW	250 ml
SD-LW agar	2500 ml

4.2.3. Required material

- » 6 pouches of SD-AHLW powder (supplied in the Media Starter Package)
- » Glass beaker (3 liter)

- » 3 glass bottles (1 liter each)
- » 2 glass bottle (500 ml)
- » 6 magnetic stirring bars
- » 50 g Bacto agar
- » 100 sterile plastic petri dishes (10 cm diameter)

4.2.4. 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 250 ml of SD-LW master medium to a 500 ml bottle and continue with Section 4.2.5
 - » Transfer 250 ml of SD-LW master medium to a 500 ml bottle and continue with Section 4.2.6
 - » Transfer 500 ml of SD-LW master medium to a 1 liter bottle and continue with Section 4.2.7
 - » Transfer 1 liter of SD-LW master medium to each of two 1 liter bottles and continue with Section 4.2.7

4.2.5. Preparation of liquid SD-L medium

1. Add 0.5 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

4.2.6. Preparation of liquid SD-LW medium

1. Autoclave the medium at 121 °C for 15 min
2. Store the autoclaved medium at room temperature

4.2.7. Preparation of SD-LW agar medium

1. Add 10 g Bacto agar to the bottle containing 500 ml SD-WL master medium and 20 g Bacto agar to each bottle containing 1 liter of SD-WL master medium
2. Add a magnetic stirring bar to each bottle
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 100 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

4.3. SD-trp-leu-his or SD-trp-leu-his-ade agar

4.3.1. Introduction

In this section, the selective plates required for Protocol 9 (Confirmation of positive interactors) in the DUALmembrane or DUALhunter system are prepared. The type of selective medium to prepare depends on the selective conditions you have applied in the screen, according to Protocol 6 of the DUALmembrane or DUALhunter manuals.

4.3.2. Amounts to be prepared for 100 preys

Selective medium	Amounts required for 100 preys
As determined in Protocol 6	100 petri dishes (10 cm diameter) 2.5 liter selective medium

4.3.3. Required material

- » 5 pouches of SD-AHLW powder (supplied in the Media Starter Package)
- » 3-AT (supplied in the Media Starter Package)
- » 1 glass beaker (3 liter)
- » 3 glass bottles (1 liter)
- » 4 Magnetic stirring bars
- » 100 petri dishes (10 cm diameter)

4.3.4. Preparation of selective medium for one screen

1. Add the contents of the SD-AHLW pouches to the glass beaker
2. Dissolve the contents in 2.5 liters of water
3. **If your selective medium is SD-HLW (with or without 3-AT),** add 12.5 ml adenine stock solution. **If your selective medium is SD-AHLW (with or without 3-AT),** omit this step
4. Aliquot the medium into the glass bottles according to the table below:

Bottle	Volume of medium to add
A	500 ml
B	1000 ml
C	1000 ml

5. Add Bacto agar according to the Table below:

Bottle	Bacto agar to add
A	10 g
B	20 g
C	20 g

6. Add a magnetic stirring bar
7. Autoclave the medium at 121°C for 15 min
8. Place the medium in a 50°C water bath for 1-2 hours

9. 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	0.5 ml (Bottle A) 1.0 ml (Bottles B and C)
2.5 mM	1.25 ml (Bottle A) 2.5 ml (Bottles B and C)
5.0 mM	2.5 ml (Bottle A) 5.0 ml (Bottles B and C)
7.5 mM	3.75 ml (Bottle A) 7.5 ml (Bottles B and C)
10.0 mM	5 ml (Bottle A) 10.0 ml (Bottles B and C)

10. Place the bottle on a magnetic stirrer and stir for 5-10 minutes to ensure proper mixing of all components
11. In the meantime, label 100 petri dishes (10 cm diameter) with the selection conditions
12. Pour the medium into the petri dishes
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. Media preparation for the DUALhybrid kit

5.1. Liquid 1x YPAD Medium

5.1.1. Introduction

Liquid 1x YPAD medium is used in Protocol 9 (Confirmation of positive interactors).

5.1.2. Amounts to be prepared for analysis of 100 preys

1 liter of liquid 1x YPAD medium

5.1.3. Required material

- » 1 YPAD pouch (supplied in the Media Starter Package)
- » 1 glass bottle (1 liter)

5.1.4. Preparation and storage

5. Add the entire contents of 1 YPAD pouch to a 1 liter glass bottle
6. Add 1 liter of water and shake well to dissolve large clumps
7. Autoclave at 121°C for 15 min
8. Store the medium at room temperature

5.2. SD-W and SD-LW media

5.2.1. Introduction

In this section, SD-W and SD-LW liquid media and agar plates are prepared for use with Protocols 7 (Plasmid recovery from yeast and retransformation into *E. coli*) and 8 (Bait dependency test).

5.2.2. Amounts to be prepared for the analysis of 100 preys

Medium	Amount
Liquid SD-W	250 ml
Liquid SD-LW	250 ml
SD-LW agar	2500 ml

5.2.3. Required material

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

- » 2 glass bottle (500 ml)
- » 6 magnetic stirring bars
- » 50 g Bacto agar
- » 100 sterile plastic petri dishes (10 cm diameter)

5.2.4. 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 250 ml of SD-LW master medium to a 500 ml bottle and continue with Section 5.2.5
 - » Transfer 250 ml of SD-LW master medium to a 500 ml bottle and continue with Section 5.2.6
 - » Transfer 500 ml of SD-LW master medium to a 1 liter bottle and continue with Section 5.2.7
 - » Transfer 1 liter of SD-LW master medium to each of two 1 liter bottles and continue with Section 5.2.7

5.2.5. Preparation of liquid SD-W medium

1. Add 2.5 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

5.2.6. Preparation of liquid SD-LW medium

1. Autoclave the medium at 121 °C for 15 min
2. Store the autoclaved medium at room temperature

5.2.7. Preparation of SD-LW agar medium

1. Add 10 g Bacto agar to the bottle containing 500 ml SD-WL master medium and 20 g Bacto agar to each bottle containing 1 liter of SD-WL master medium
2. Add a magnetic stirring bar to each bottle
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 100 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

5.3. SD-trp-leu-his or SD-trp-leu-his-ade agar

5.3.1. Introduction

In this section, the selective plates required for Protocol 8 (Bait dependency test) in the DUALhybrid system are prepared. The type of selective medium to prepare depends on the selective conditions you have applied in the screen, according to Protocol 6 of the DUALhybrid manual.

5.3.2. Amounts to be prepared for 100 preys

Selective medium	Amounts required for 100 preys
As determined in Protocol 6	100 petri dishes (10 cm diameter) 2.5 liter selective medium

5.3.3. Required material

- » 5 pouches of SD-AHLW powder (supplied in the Media Starter Package)
- » 3-AT (supplied in the Media Starter Package)
- » 1 glass beaker (3 liter)
- » 3 glass bottles (1 liter)
- » 4 Magnetic stirring bars
- » 100 petri dishes (10 cm diameter)

5.3.4. Preparation of selective medium for one screen

1. Add the contents of the SD-AHLW pouches to the glass beaker
2. Dissolve the contents in 2.5 liters of water
3. **If your selective medium is SD-HLW (with or without 3-AT),** add 12.5 ml adenine stock solution. **If your selective medium is SD-AHLW (with or without 3-AT),** omit this step
4. Aliquot the medium into the glass bottles according to the table below:

Bottle	Volume of medium to add
A	500 ml
B	1000 ml
C	1000 ml

5. Add Bacto agar according to the Table below:

Bottle	Bacto agar to add
A	10 g
B	20 g
C	20 g

6. Add a magnetic stirring bar
7. Autoclave the medium at 121 °C for 15 min
8. Place the medium in a 50 °C water bath for 1-2 hours

9. 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	0.5 ml (Bottle A) 1.0 ml (Bottles B and C)
2.5 mM	1.25 ml (Bottle A) 2.5 ml (Bottles B and C)
5.0 mM	2.5 ml (Bottle A) 5.0 ml (Bottles B and C)
7.5 mM	3.75 ml (Bottle A) 7.5 ml (Bottles B and C)
10.0 mM	5 ml (Bottle A) 10.0 ml (Bottles B and C)

10. Place the bottle on a magnetic stirrer and stir for 5-10 minutes to ensure proper mixing of all components
11. In the meantime, label 100 petri dishes (10 cm diameter) with the selection conditions
12. Pour the medium into the petri dishes
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

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