CloneDetect

Detection Reagent

Protocol User Guide

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MOLECULAR DEVICES

Product manufactured by Molecular Devices, LLC.
3860 N. First Street, San Jose, California, 95134, United States of America.
Molecular Devices, LLC is ISO 9001 registered.

Contents

Chapter 1: CloneDetect Detection Reagents

The CloneDetect detection reagents are specifically designed for use in immunoglobulin productivity assays with semi-solid media on ClonePix® systems. CloneDetect binds and precipitates the secreted antibody product around producing colonies in the semi-solid media, thus generating a localized fluorescent signal that can be imaged and quantified on ClonePix 2 systems.

Under optimized assay conditions, the accumulated fluorescent signal is proportional to the amount of protein secreted by a given clone and allows reliable identification of high-producing cell lines.

CloneDetect products are antibody-based, fluorescent detection agents which have been specifically developed for this purpose.

CloneDetect products are available in formulations to suit:

- Transfected cell line assays for humanized antibodies (for example, myeloma, CHO, HEK293, PER.C6).
- Hybridoma assays detecting mouse-derived antibodies.

Key properties of CloneDetect Anti-Mouse and Anti-Human detection agents are:

- Exceptional brightness of signal
- Low levels of background
- High target specificity
- Stability in semi-solid culture media over prolonged periods of culture
- Cell-culture validated, sterile and azide-free
- Regulatory friendly and safe to use on cell substrates
- Simple, unit concentrations for optimized performance on ClonePix Systems
- Compatible with other cell analyzer instrument with green fluorescent channels (for example, Flow Cytometry (FACs), CloneSelect® Single-Cell Printer (f.sight), and CloneSelect Imager FL).

For applications required to satisfy highest regulatory demands, Recombinant CloneDetect offers a unique recombinant alternative. Recombinant CloneDetect is a fluorescein labeled recombinant monoclonal antibody molecule (mouse IgG), which are produced by engineered CHO cells. The product has a high specificity and affinity for human IgG Fc region. Moreover, each batch has been subjected to a rigorous regimen of safety assessment: encompassing testing for the presence of both mycoplasma and a panel of adventitious viral agents, and the analysis of endotoxin levels.

Choose the appropriate protocol for your application:

Protocol	Description
Protocol A	Using CloneDetect with CloneMatrix and Medium of your Choice For CHO, HEK293, PER.C6, myeloma, stem cell and Hybridoma cells: • CloneDetect Anti-Human products: K8202, K8205, K8400, K8495 (recombinant) • CloneDetect Anti-Mouse products: K8420, K8425 • CloneMatrix products: K8500, K8510, K8520, K8530 • CloneXL CHO animal-component-free (ACF) supplement reagent product (50x concentrated): K8525 • XP Media CHO Growth A liquid medium animal-component-free product: K8860 CloneMatrix is developed specifically for optimized use with ClonePix systems. It supports colony formation, as well as fluorescent assays, using CloneDetect Agent. CloneMatrix is free from animal-sourced products and suitable for users wishing to work under chemically-defined conditions or for use with CloneXL, XP Media CHO Growth A, and Recombinant CloneDetect to satisfy highest regulatory demands.
Protocol B	Using CloneDetect with CloneMedia for CHO and HEK293 cells: • CloneDetect Anti-Human products: K8202, K8205, K8400, K8495 (recombinant) • CloneMedia semi-solid medium products: K8680, K8685, K8800, K8810, K8830, K8840 • XP Media CHO Growth A liquid medium animal-component-free product: K8860

Storage and Handling

- Store all CloneDetect products at between 2°C and 8°C (35.6°F and 46.4°F).
- Always protect CloneDetect products from prolonged exposure to direct light.
- CloneDetect will remain stable for 12 months from shipment of product when stored in these conditions.
- Store all semi-solid media products at -20°C (-4°F) or lower.
- Sterile culture environment and ASEPTIC technique are required when handling the reagents described in this guide.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website—www.moleculardevices.com/service-support—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Technical Support

To contact Molecular Devices Technical Support, submit a support request through the Molecular Devices Knowledge Base at support.moleculardevices.com.

You can also submit a support request by phone. For regional support contact information, go to www.moleculardevices.com/contact.

To expedite support, have the following information available when you call:

- Product Name
- Part Number
- Sales Order Number
- Lot Number

Chapter 2: Protocol A - Using CloneDetect with CloneMatrix and a Medium of Your Choice

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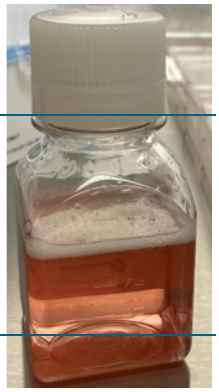
1. Completely thaw a 40 ml (1.35 oz) bottle of CloneMatrix at between 2°C and 8°C (35.6°F and 46.4°F) overnight or at room temperature for 2 to 3 hours.

   CAUTION! Do not thaw CloneMatrix in a 37°C (98.6°F) water bath. Doing this can prevent the semi-solid media made from solidifying properly.

2. After complete thawing, vigorously shake the bottle for 2 minutes to thoroughly mix the contents.

   CAUTION!
   - Do not shake the bottle if ice is still present.
   - Be sure to vigorously shake the bottle. Inadequate mixing—for example, shaking the bottle a few times or swirling it gently—can prevent the semi-solid media from solidifying properly.
   - Do not use serological pipettes to mix the contents due to the high viscosity of the contents.
   - Mix the whole bottle and allow the mixture to de-gas before making aliquots.
   - Use a positive displacement pipette or a syringe for accurate pipetting.
   - If you do not plan to use the thawed media immediately, you can store in a refrigerator. However, you must again vigorously shake the bottle and allow it to adjust to room temperature before use.

3. After vigorously shaking the bottle, air bubbles will form. To clear the bubbles, let the bottle stand at room temperature for 20 minutes or until all the bubbles dissipate (as shown to the right).



   CAUTION!
   - CloneMatrix must be at room temperature before you add any components.
   - Do not warm CloneMatrix in a 37°C (98.6°F) water bath. Instead, let the bottle stand at room temperature for 20 minutes.

4. Prepare 10 ml complete semi-solid media containing all necessary media, antibiotics, supplements, and selection agent components as per manufacturer's instructions. To do this, do one of the following:
   - Add the following components in a sterile 14 ml polypropylene tube (Fisher #14-959-11B or equivalent):
     - 4.0 ml CloneMatrix (K8500, K8510, K8520, K8530)
     - 5.0 ml 2x concentrated medium of your choice
     - 0.0 ml to 0.4 ml Gibco 200mM L-Glutamine (Fisher #25-030-081 or equivalent)
     - 0.0 ml to 0.2 ml CloneXL CHO ACF supplement (K8525), optimized to grow CHO cells
     - 0.2 ml to 0.8 ml other components (antibiotics, supplements, selection agents, media)

     The total final volume is 9.8 ml.
   - If using a 4x concentrated medium of your choice, add the following components in a sterile 14 ml polypropylene tube (Fisher #14-959-11B or equivalent):
     - 4.0 ml CloneMatrix (K8500, K8510, K8520, K8530)
     - 2.5 ml 4x concentrated medium of your choice
     - 0.0 ml to 0.4 ml Gibco 200mM L-Glutamine (Fisher #25-030-081 or equivalent)
     - 0.0 ml to 0.2 ml CloneXL CHO ACF supplement (K8525), optimized to grow CHO cells
     - 2.7 ml to 3.3 ml other components (antibiotics, supplements, selection agents, media)

     The total final volume is 9.8 ml.
   - If you want fast media solidification, add the following components in a sterile 14 ml polypropylene tube (Fisher #14-959-11B or equivalent):
     - 4.5 ml CloneMatrix (K8500, K8510, K8520, K8530)
     - 2.5 ml 4x concentrated medium of your choice
     - 0.0 ml to 0.4 ml Gibco 200mM L-Glutamine (Fisher #25-030-081 or equivalent)
     - 0.0 ml to 0.2 ml CloneXL CHO ACF supplement (K8525)
     - 2.2 ml to 2.8 ml other components (antibiotics, supplements, selection agents, media)

     The total final volume is 9.8 ml.

   The CloneMatrix-CHO (K8520, K8530) kit contains CloneMatrix and CloneXL CHO ACF supplement (50x concentrated).
   - Store the CloneXL CHO ACF supplement reagent at -20°C (-4°F).
   - Thaw the CloneXL CHO ACF supplement reagent at 4°C (-39.2°F) overnight or at room temperature just prior to use.
   - After thawing, allow the CloneXL CHO ACF supplement reagent to adjust to room temperature prior to use.

Note: Due to the high viscosity of CloneMatrix, we recommend using a blunt end sterile syringe to accurately measure the CloneMatrix volume.

5. Add 100 µl CloneDetect (provided at a 10,000 U/ml concentration) per 10 ml of complete semi-solid media.

   Note:
   - One full vial, which is 1 ml of detection agent, is sufficient for 100 ml of semi-solid media.
   - Some cell lines may require further optimization. For best results, you may need to determine the concentration of CloneDetect empirically. This is particular important for recombinant monoclonal CloneDetect (K8495), which is highly specific for human IgG Fc region antibody detection.

6. Invert the tube 8 to 10 times and mix gently to distribute CloneDetect evenly throughout the media.

   CAUTION! CloneDetect is photosensitive. To avoid photobleaching, always cover the media and protect it from direct light.

7. Add cell suspensions (50 µl to 100 µl) per 10 ml of complete semi-solid media for pre-optimized seeding density.

   Note: The optimal cell number should be determined empirically. Refer to the protocol supplied with the semi-solid media product for detailed instructions on seeding density optimization.

   Tip: Always include an appropriate cell line that is known to secrete mouse or human IgG antibodies (depending on the CloneDetect used) as a positive control to run in parallel with an unknown sample. For example:
   - SJK-287-38 cell line secreting mouse IgG antibodies from ECACC Cat# 90112228 or ATCC Cat# CRL-1644
   - B13-24 cell line secreting human IgG antibodies from ATCC Cat# CRL-11397

8. Invert the tube 8 to 10 times to gently distribute the cells evenly throughout the semi-solid media.
9. Do one of the following to dispense the semi-solid media:

   For ClonePix 2:
   - Dispense 1.5 to 2.0 ml per well into a 6-well plate (Greiner #657185 or Nunc #140675) or prepare 15 to 20 ml per OmniTray (Nunc Omni #242811). To ensure equal amounts of semi-solid media per well for a 6-well plate, gently tip the tube until drops start to fall slowly and steadily. Pour 5 drops (about 1 ml) per well, then add 1 drop per well until each well contains 8 to 10 drops (about 1.5 to 2.0 ml).

   For ClonePix 2 enhanced (with monoclonality assurance):
   - Dispense 1 ml (about 4 to 5 drops) per well into a 6-well plate (Greiner #657185).

10. Tilt and rotate the plate (only on Day 0) to ensure the entire surface of the wells or the single-well OmniTray is covered with semi-solid media.

    CAUTION! Confirm that the wells are completely covered. This is particularly important if you are using ClonePix 2 enhanced (with monoclonality assurance).

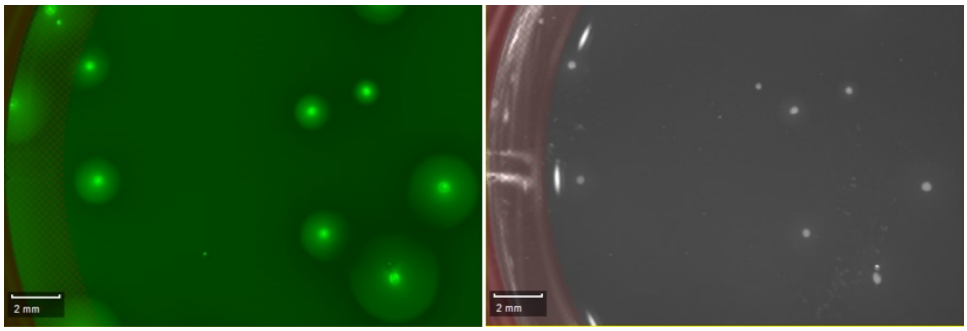
11. If you are using ClonePix 2 enhanced (with monoclonality assurance), wait 1 to 4 hours to allow the cells to settle and the media to solidify before performing Day 0 imaging.

    Note: You can speed the time for settlement and solidification, you can centrifuge the plate at 1000 rpm for 5 minutes at room temperature.

12. Incubate seeded plates at 37°C (98.6°F) and 5% CO_2 with a humidity of 75% or higher.
13. **Leave cultures undisturbed for at least 4 days**. If necessary, check for media solidification or optimize the time point for plate imaging. We recommend using an additional seeded plate.

    CAUTION!
    - DO NOT tilt or rotate the plate after Day 0. ALWAYS keep the plate level.
    - If you use a water pan to control humidity, be sure to have at least 2.5 cm (1 in.) of sterile water in the water pan during the entire incubation time.

14. After semi-solid media solidifies, do one of the following to prevent it from drying out:
    - Fill the space between wells with 4 ml cell culture grade water if using a 6-well plate.
    - Leave the culture plates in a wet box with humidity control.
15. Verify the presence of growing colonies at a suitable time point using a light microscope or CloneSelect imager.
16. Image plates on a ClonePix system. (See the ClonePix documentation on the Molecular Devices Knowledge Base at support.moleculardevices.com for details.)



Mouse CloneDetect K8425 was directly added to SJK-287-38 cells (ECACC No. 90112228) semi-solid culture on Day 0. The 6-well plate was imaged on a ClonePix system on Day 7 post plating under FITC channel (on the left) and white light channel (on the right).

Chapter 3: Protocol B - Using CloneDetect with CloneMedia

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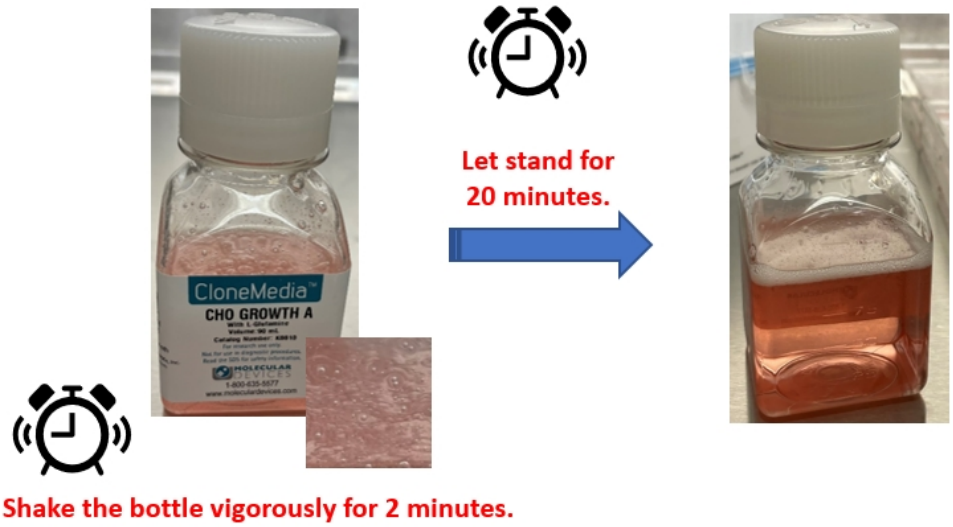
1. Completely thaw a 90 ml (3 oz) bottle of CloneMedia at between 2°C and 8°C (35.6°F and 46.4°F) overnight or at room temperature for 2 to 3 hours

CAUTION! Do not thaw the bottle in a 37°C (98.6°F) water bath; doing this can prevent the semi-solid media made from solidifying properly.

2. After complete thawing, vigorously shake the bottle for 2 minutes to thoroughly mix the contents.

CAUTION!

- Do not shake the bottle if ice is still present.
- Be sure to vigorously shake the bottle. Inadequate mixing—for example, shaking the bottle a few times or swirling it gently—can prevent the semi-solid media from solidifying properly.
- Do not use serological pipettes to mix the contents due to the high viscosity of the contents.
- Mix the whole bottle and allow the mixture to de-gas before making aliquots.
- Use a positive displacement pipette or a syringe for accurate pipetting.
- If you do not plan to use the thawed media immediately, you can store in a refrigerator. However, you must again vigorously shake the bottle and allow it to adjust to room temperature before use.



3. After vigorously shaking the bottle, air bubbles will form. To clear the bubbles, let the bottle stand at room temperature for 20 minutes or until all the bubbles dissipate (as shown above).

CAUTION!

- CloneMedia must be at room temperature before you add any components.
- Do not warm CloneMedia in a 37°C (98.6°F) water bath. Instead, let the bottle stand at room temperature for 20 minutes.

4. Prepare 10 ml complete semi-solid media containing all necessary media, antibiotics, supplements, and selection agent components as per manufacturer's instructions. To do this, do one of the following:
   - Add the following components in a sterile 14 ml polypropylene tube (Fisher #14-959-11B or equivalent):
     - 9.0 ml CloneMedia (K8680, K8685, K8800, K8810, K8830, K8840)
     - 0.0 ml to 0.4 ml Gibco 200 mM L-Glutamine (Fisher # 25-030-081 or equivalent)
     - 0.0 ml to 0.2 ml CloneXL CHO ACF supplement (K8525), optimized to grow CHO cells
     - 0.2 ml to 0.8 ml other components (antibiotics, supplements, selection agents, media)

     The total final volume is 9.8 ml.
   - If you want fast media solidification for a concentrated protocol, add the following components in a sterile 14 ml polypropylene tube (Fisher #14-959-11B or equivalent):
     - 9.5 ml CloneMedia (K8800, K8810)
     - 0.0 ml to 0.2 ml CloneXL CHO ACF supplement (K8525), optimized to grow CHO cells
     - 0.1 ml other components (antibiotics, supplements, selection agents, media)

     The total final volume is 9.8 ml.
   - If you want fast media solidification for an enhanced protocol, add the following components in a sterile 14 ml polypropylene tube (Fisher #14-959-11B or equivalent):
     - 9.0 ml CloneMedia (K8800, K8810)
     - 0.5 ml CloneMatrix (K8500, K8510, K8520, K8530)
     - 0.0 ml to 0.2 ml CloneXL CHO ACF supplement (K8525), optimized to grow CHO cells
     - 0.1 ml other components (antibiotics, supplements, selection agents, media)

     The total final volume is 9.8 ml.

Note:
- Due to the high viscosity of CloneMedia, we recommend using a blunt end sterile syringe to accurately measure the CloneMedia volume.
- We recommend adding CHO growth A liquid Media (K8860) if needed to achieve the final desired volume for growing CHO cells.

5. Add 100 µl CloneDetect (provided at a 10,000 U/ml concentration) per 10 ml of complete semi-solid media.

Note:
- One full vial, which is 1 ml of detection agent, is sufficient for 100 ml of semi-solid media.
- Some cell lines may require further optimization. For best results, you may need to determine the concentration of CloneDetect empirically. This is particular important for recombinant monoclonal CloneDetect (K8495), which is highly specific for human IgG Fc region antibody detection.

6. Invert the tube 8 to 10 times and mix gently to distribute CloneDetect evenly throughout the media.

CAUTION! CloneDetect is photosensitive. To avoid photobleaching, always cover the media and protect it from direct light.

7. Add cell suspensions (50 µl to 100 µl) per 10 ml of complete semi-solid media for pre-optimized seeding density.

   Note: The optimal cell number should be determined empirically. Refer to the protocol supplied with the semi-solid media product for detailed instructions on seeding density optimization.

   Tip: Always include an appropriate cell line that is known to secrete mouse or human IgG antibodies (depending on the CloneDetect used) as a positive control to run in parallel with an unknown sample. For example:
   - SJK-287-38 cell line secreting mouse IgG antibodies from ECACC Cat# 90112228 or ATCC Cat# CRL-1644
   - B13-24 cell line secreting human IgG antibodies from ATCC Cat# CRL-11397

8. Invert the tube 8 to 10 times to gently distribute the cells evenly throughout the semi-solid media.
9. Do one of the following to dispense the semi-solid media:

   For ClonePix 2:
   - Dispense 1.5 to 2.0 ml per well into a 6-well plate (Greiner #657185 or Nunc #140675) or prepare 15 to 20 ml per OmniTray (Nunc Omni #242811). To ensure equal amounts of semi-solid media per well for a 6-well plate, gently tip the tube until drops start to fall slowly and steadily. Pour 5 drops (about 1 ml) per well, then add 1 drop per well until each well contains 8 to 10 drops (about 1.5 to 2.0 ml).

   For ClonePix 2 enhanced (with monoclonality assurance):
   - Dispense 1 ml (about 4 to 5 drops) per well into a 6-well plate (Greiner #657185).
10. Tilt and rotate the plate (only on Day 0) to ensure the entire surface of the wells or the single-well OmniTray is covered with semi-solid media.

    CAUTION! Confirm that the wells are completely covered. This is particularly important if you are using ClonePix 2 enhanced (with monoclonality assurance).

11. If you are using ClonePix 2 enhanced (with monoclonality assurance), wait 1 to 4 hours to allow the cells to settle and the media to solidify before performing Day 0 imaging.

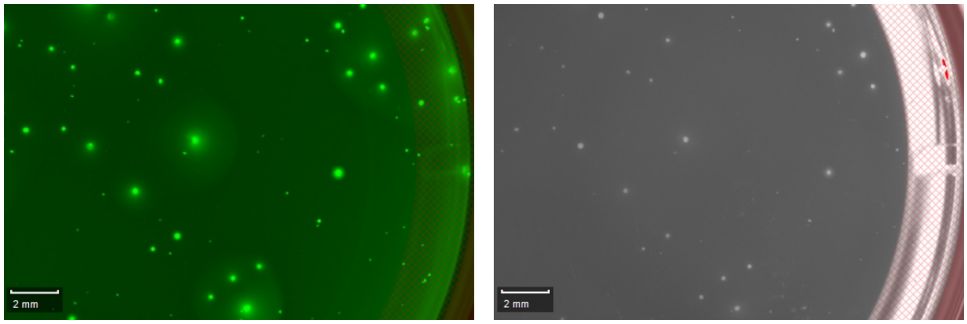
    Note: You can speed the time for settlement and solidification, you can centrifuge the plate at 1000 rpm for 5 minutes at room temperature.

12. Incubate seeded plates at 37°C (98.6°F) and 5% CO_2 with a humidity of 75% or higher.
13. **Leave cultures undisturbed for at least 4 days**. If necessary, check for media solidification or optimize the time point for plate imaging. We recommend using an additional seeded plate.

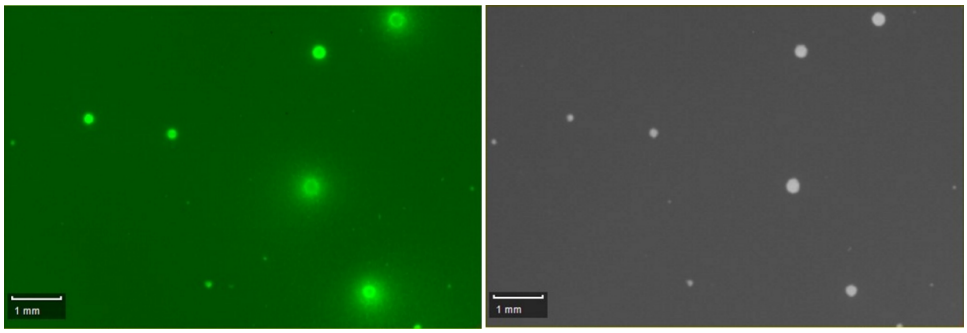
    CAUTION!
    - DO NOT tilt or rotate the plate after Day 0. ALWAYS keep the plate level.
    - If you use a water pan to control humidity, be sure to have at least 2.5 cm (1 in.) of sterile water in the water pan during the entire incubation time.

14. After semi-solid media solidifies, do one of the following to prevent it from drying out:
    - Fill the space between wells with 4 ml cell culture grade water if using a 6-well plate.
    - Leave the culture plates in a wet box with humidity control.

15. Verify the presence of growing colonies at a suitable time point using a light microscope or CloneSelect imager.
16. Image plates on a ClonePix system. (See the ClonePix documentation on the Molecular Devices Knowledge Base at support.moleculardevices.com for details.)



Human polyclonal CloneDetect K8400 was directly added to a semi-solid culture of the transfected CHO DG44 cells secreting human IgG1 antibodies on Day 0. The 6-well plate was imaged on a ClonePix system on Day 14 post plating under FITC channel (on the left) and white light channel (on the right).



Human recombinant monoclonal CloneDetect K8495 was directly added to a semi-solid culture of the transfected CHO DG44 cells secreting human IgG1 antibodies on Day 0. The 6-well plate was imaged on a ClonePix system on Day 14 post plating under FITC channel (on the left) and white light channel (on the right).

Appendix A: Ordering CloneDetect Detection Reagents

To reorder reagents, go to:

- shop.moleculardevices.com (North America only)
- www.moleculardevices.com/quote-request (Outside of North America)

Product List

Symbol	Description	Measurements
K8202	CloneDetect anti-human detection agent, polyclonal, FITC label, Sterile, BSA free; azide free	10,000 U/ml
K8205	CloneDetect anti-human detection agent, Fc Gamma chain specific, polyclonal, FITC label, Sterile, azide-free	10,000 U/ml
K8400	CloneDetect anti-human detection agent, polyclonal, FITC label, Sterile, azide-free	10,000 U/ml
K8420	CloneDetect anti-mouse detection agent, polyclonal, FITC label, Sterile, azide free	10,000 U/ml
K8425	CloneDetect anti-mouse detection agent, Fc Gamma fragment specific, polyclonal, FITC label, Sterile, azide free	10,000 U/ml
K8495	Recombinant CloneDetect anti-human, Fc region specific, monoclonal, FITC label, Sterile, BSA-free, azide-free, animal origin-free, low endotoxin level	10,000 U/ml

Note:

- 1 unit = optimized amount of detection agent required per 10 µl of semi-solid media.
- For K8495, the required concentration may be dependent on the cell line. For best results, you may need to determine the concentration of CloneDetect empirically.

Related Media and Supplement

Symbol	Description
K8500	CloneMatrix, makes 6 × 100 ml final media volume 6 × 40 ml
K8510	CloneMatrix, makes 100 ml final media volume 1 × 40 ml
K8520	CloneMatrix-CHO: optimized for CHO cells, contains 6x 2 ml CloneXL and 6 × 40 ml CloneMatrix to make 6 × 100 ml final media when use 2x concentrated media of your choice
K8525	CloneXL reagent, 50x concentrated, CHO ACF supplement (animal-component-free), converts CloneMatrix to CloneMatrix-CHO 5 × 2 ml
K8530	CloneMatrix-CHO: optimized for CHO cells, contains 1x 2mL CloneXL ACF supplement and 1x 40 ml CloneMatrix to make 100 ml final media when use 2x concentrated media of your choice
K8680	CloneMedia-HEK (semi-solid media for HEK cells) 6 × 90 ml
K8685	CloneMedia-HEK (semi-solid media for HEK cells) 1 × 90 ml
K8800	CloneMedia CHO Growth A with L-Gln 6 × 90 ml
K8810	CloneMedia CHO Growth A with L-Gln 1 × 90 ml
K8830	CloneMedia CHO Growth A without L-Gln 6 × 90 ml
K8840	CloneMedia CHO Growth A without L-Gln 1 × 90 ml
K8860	XP Media CHO Growth A without L-Gln 1000 ml

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