gibco

CTS™ DynaMag™ Magnet

For optimal separation of Dynabeads™ magnetic beads

Catalog Number 12102

Document Part Number 4466509

Publication Number MAN0008032

Revision C.0

For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. CAUTION: Not intended for direct administration into humans or animals.

ThermoFisher SCIENTIFIC

Manufactured by OEM partner for Life Technologies A.S. | Ullernchausseen 52 PO Box 114, Smestad | Oslo, Norway N-0379
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0008032

Revision	Date	Description
C.0	5 May 2021	Update of illustrations and layout. Revision of protocols for clarity.
B.0	6 March 2019	Rebrand update and change of product name.
A.0	25 May 2015	Baseline for revision.

Contents

1 Product information

Product description

The CTS™ DynaMag™ Magnet is a magnetic device for use in combination with Dynabeads™ magnetic beads for medium to large-scale separation of cells.

The magnet is suitable for use with commercially available sterile blood/culture bags, tubing and connectors to perform positive isolation of target cells or depletion of unwanted cells in a sterile and closed system.

The CTS™ DynaMag™ Magnet is intended for research use or manufacturing of cell, gene, or tissue-based products.

The magnet can be used in combination with clinical research products from the CTS™ Dynabeads™ portfolio to:

- Positively isolate bead-bound cells for subsequent stimulation/expansion of T cells with CTS™ Dynabeads™ magnetic beads, then remove Dynabeads™ magnetic beads after expansion.
- Deplete unwanted cell types by discarding magnetically captured bead-bound cells with customized Dynabeads™ products for depletion of specific cell populations.

Storage

Store at ambient temperature. Storage at this temperature does not lead to decrease in magnetism. Protect the CTS™ DynaMag™ Magnet from vibration and keep out of direct sunlight.

System overview

CAUTION! This device contains extremely powerful permanent magnets. Keep ferromagnetic and ferromagnetically-sensitive material away from the magnetic surfaces and associated fields. Do not bring tools, equipment or personal items containing steel, iron or other magnetic materials close to the magnets. The strong magnetic field can erase magnetic media such as USB memory devices, disable ATM and credit cards, and can damage some watches. Strong magnetic fields can also cause serious injury to persons with implanted or attached medical devices, such as pacemakers and prosthetic parts.

The Health and Safety Officer should take all necessary steps and full responsibility to ensure that the precautions and statements are followed and adhered to. IN NO EVENT SHALL THERMO FISHER SCIENTIFIC AS BE LIABLE FOR ANY SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES.

The CTS™ DynaMag™ Magnet consists of a Rotation Unit with a detachable Primary Magnet, a Secondary Magnet, a Base Unit with a Solution Pole to hold a bag with priming and washing solution, and a retractable plate to hold the bag with the residual cells after the magnetic capture of bead-bound

cells. Standard blood bags and tubing are included in the figure for illustrative purposes only, and are not supplied by Thermo Fisher Scientific.

See "Operating the CTS™ DynaMag™ Magnet" on page 12 for detailed information of the operation of the CTS™ DynaMag™ Magnet.

Description of parts

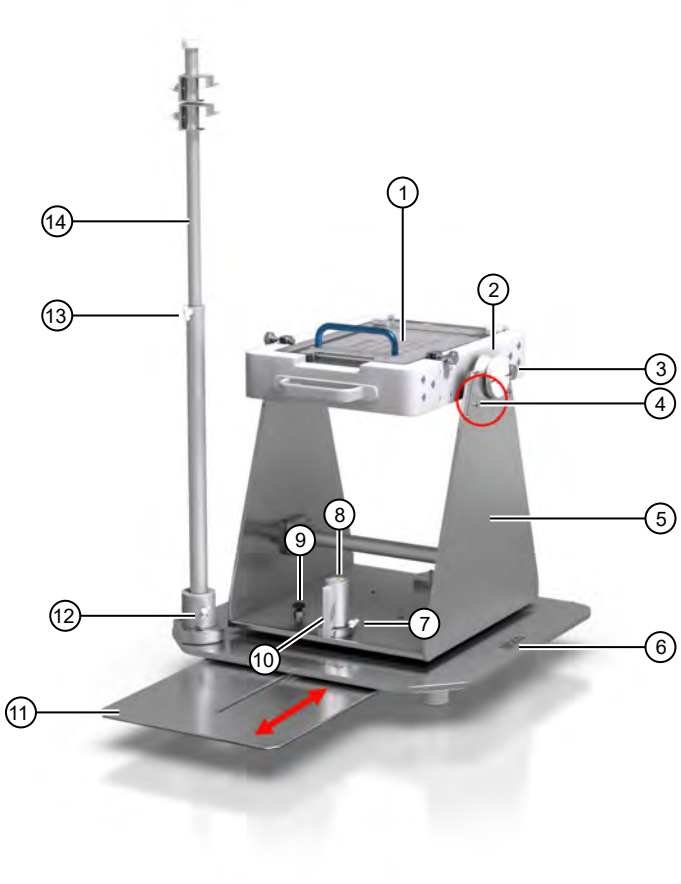


Figure 1 Detailed overview of the CTS™ DynaMag™ Magnet

1. Primary Magnet
2. Roller
3. Magnet Fixing Pin
4. Magnet Angle Gauge
5. Rotation Unit
6. Base Unit
7. Set screw (Secondary Magnet)
8. Secondary Magnet (Covered with Protective Cap)
9. Locking Pin
10. Tube Gripper
11. Retractable Plate
12. Set screw (Solution Pole socket)
13. Set screw (Solution Pole)
14. Solution Pole

Primary Magnet

The Primary Magnet consists of an array of extremely strong permanent Neodymium-Iron-Boron magnets. The arrangement is optimized to give high field strength and a favorable field gradient, which ensures efficient separation of M-450 Dynabeads™ magnetic beads within 1 minute. Optimal separation takes place in closed, sterile bags at an approximate distance of 11 mm or less from the magnet surface, which is possible with sample volumes up to 330 mL in 1000 mL bags in static separations. A continuous-flow separation procedure for larger volumes using an equivalent magnetic separation system is described.

The Primary Magnet can be detached from the Rotation Unit to allow refrigeration if cold separations are desired. See “Operating the CTS™ DynaMag™ Magnet” on page 12 for the detachment/attachment procedures.

The Primary Magnet can be inclined stepwise to optimize separation (Figure 3 on page 8).

The Primary Magnet is protected by a transparent plexiglass lid. Stainless steel plates embedded in the lid apply pressure to the sample bag by magnetic attraction. In addition, springs in the Lid Shutting Pin unit will help to compress the bag. These features ensure that the Dynabeads™ magnetic beads are kept within the optimal range of the magnetic field.

Note: The field strength of the magnet decreases exponentially with the distance to the surface of the magnet. If the field strength is found to be too high for a specific application, adjust the distance to the magnet by adding a thin spacer (e.g., plastic film or a bench coat, between the bag and the magnet).

Figure 2 CTS™ DynaMag™ Primary Magnet

1. Lid
2. Stainless Steel Plate
3. Magnet Grip
4. Roller
5. Shutting Pin
6. Open/Close Handle

The Primary Magnet can be positioned below or above the bag by combining rotation of the base with inverting the Primary Magnet.

The magnet can be inclined counterclockwise at –15°, 0°, 15°, 30°, 45°, 60°, 90°, 165°, 180°, and 195° to optimize the separation. Set the angle by turning the magnet to the required angle indicated on the Angle Gauge and set the Magnet Fixing Pin.

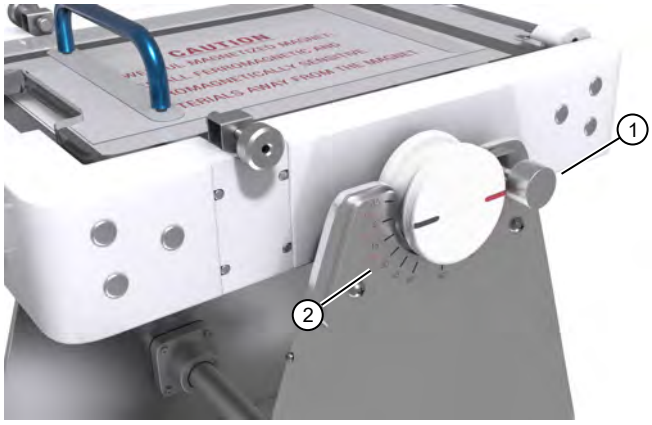


Figure 3 Adjusting the angle of the CTS™ DynaMag™ Primary Magnet

① Magnet Fixing Pin
② Magnet Angle Gauge

Secondary Magnet

The pillar shaped Secondary Magnet is used to trap residual Dynabeads™ magnetic beads that may escape the Primary Magnet, and can be fixed to the Rotation Unit in two positions depending on the configuration of the Primary Magnet (see “Change the configuration of the Primary Magnet” on page 26).

The Secondary Magnet is formed from individual Neodymium-Iron-Boron magnets oriented parallel to the axis of the pillar.

For details on setting up the Secondary Magnet, see “Set up the Secondary Magnet” on page 15.

Place the Protective Cap over the Secondary Magnet when it is not in use.

Figure 4 Tubing wrapped four times around the Secondary Magnet

① Outlet tubing
② Secondary magnet
③ Tube Gripper

Base Unit

The Base Unit supports the Rotation Unit and a Solution Pole for the bag with priming and washing solution. A Retractable Plate under the front of the base provides extra space for another bag (see Figure 1 on page 6).

Rotation Unit

The Rotation Unit can be rotated 0°, 90° and 180°. To release the Rotation Unit, pull up the Locking Pin (see Figure 3 on page 8). The pin will automatically fix the Rotation Unit at the desired position.

Retractable Plate

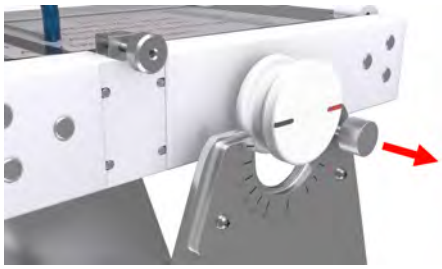
The Retractable Plate under the Base Unit provides additional space and can hold a bag of up to 3 kg (see Figure 1 on page 6). Placing the bag horizontally is considered to be gentler to the cells.

Solution Pole

The Solution Pole supports the bag with priming and washing solution. The height of the pole can be adjusted from 50 to 80 cm (see Figure 1 on page 6).

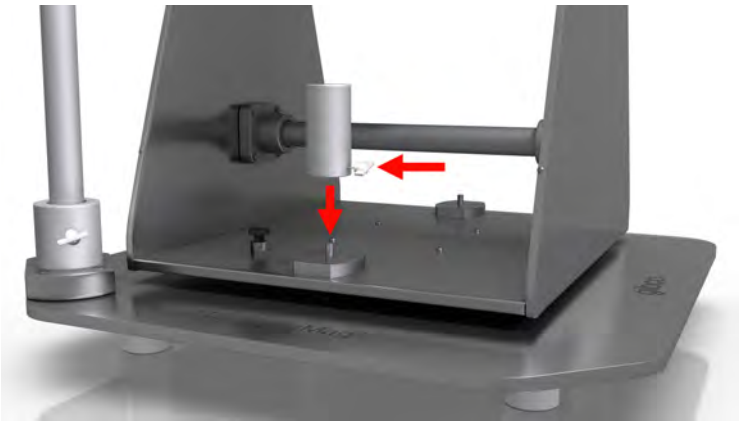
Set up the CTS DynaMag Magnet

1. Pull out the Magnet Fixing Pin on the side of the Rotation Unit.
2. Place the Primary Magnet onto the Rotation Unit by fitting the grooves on the Rollers onto the Roller Guides.

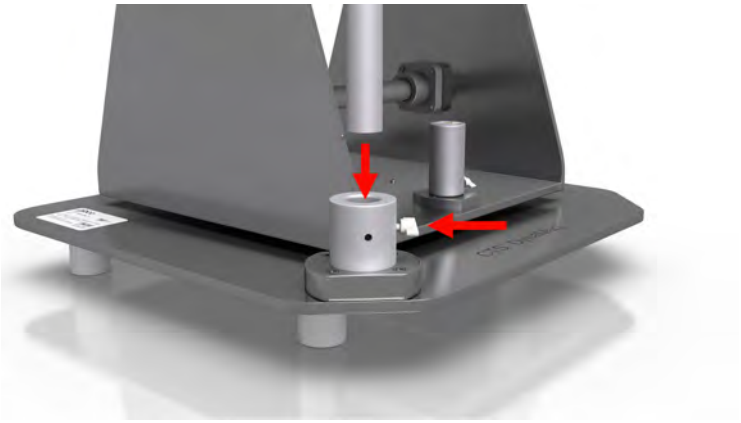


3. Push the Magnet Fixing Pin to lock the Primary Magnet in place.

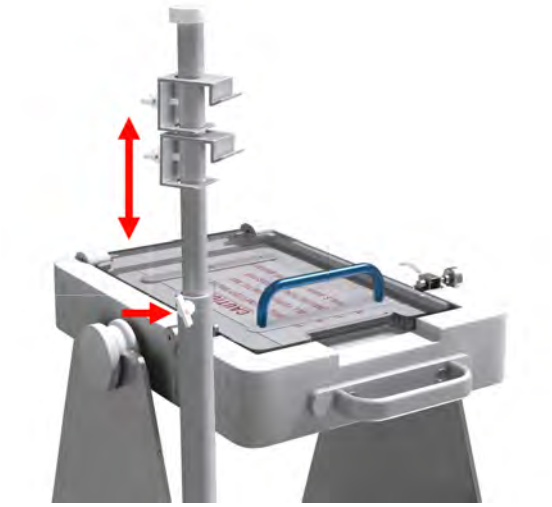
4. Place the Secondary Magnet on the locator pin on the Rotation Unit and tighten the set screw.



5. Place the Solution Pole into the socket on the Base Unit and tighten the set screw.



6. Loosen the set screw to adjust the height of the Solution Pole as necessary, then tighten the set screw to lock the pole at the set height.



Required materials not supplied

See Appendix D, “Ordering information” for details.

- Digital Rocker
- Labtainer™ BioProcess Container (BPC)
- CTS™ Dynabeads™ CD3/CD28
- Dynabeads™ Human T-Expander CD3/CD28
- CTS™ Dynabeads™ Treg Xpander
- CTS™ DPBS without calcium chloride, without magnesium chloride
- CTS™ OpTmizer™ T-Cell Expansion SFM
- CTS™ OpTmizer™ T-Cell Expansion SFM, no phenol red
- CTS™ OpTmizer™ Pro Serum Free Medium (SFM), No Phenol Red
- CTS™AIM-V™ Medium
- CTS™ Immune Cell SR
- RIPA Lysis and Extraction Buffer
- DNase I
- Proteinase K Solution (20 mg/mL), RNA grade

Operating the CTS™ DynaMag™ Magnet

The Primary Magnet can be detached from the Rotation Unit for refrigeration prior to the cell separation if cold conditions are required. Prior to detaching/attaching the Primary Magnet, remove the Solution Pole from the Base Unit.

As the Primary Magnet is heavy, it is easier to lift the magnet by its grips (Figure 2) if the rotation unit is positioned perpendicular to the base position. Turn the Rotation Unit 90˚as shown in Figure 3).

Ensure that the Magnet Fixing Pin is released (see page 14).

Attach the Primary Magnet

1. Pull out the Magnet Fixing Pin on the side of the Rotation Unit.
2. Hold the Primary Magnet grips firmly, then place the Primary Magnet onto the Rotation Unit by fitting the grooves on the Rollers onto the Roller Guides.

3. Push the Magnet Fixing Pin to lock the Primary Magnet in place.

Detach the Primary Magnet

1. Release the Magnet Fixing Pin.

2. Hold the Primary Magnet grips firmly, then pull the Primary Magnet straight up to remove it the from the Rotation Unit.

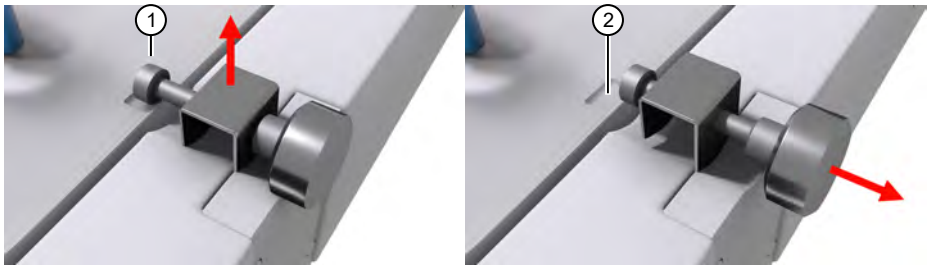
3. Push the Magnet Fixing Pin back in place.

Open and close the Primary Magnet Lid

Open the Primary Magnet Lid using the following procedure. Close the Primary Magnet Lid by reversing the procedure.

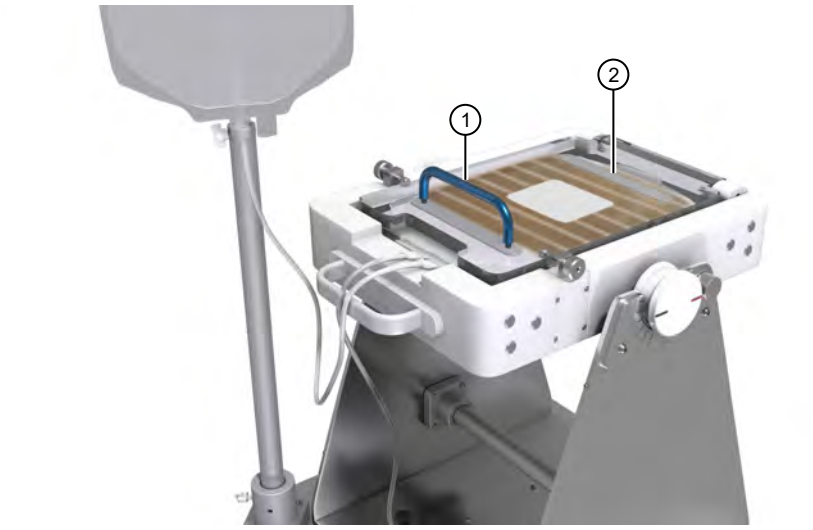
1. Lift the Shutting Pin and remove the pinhead from the groove in the lid.

2. Pull out the Shutting Pin.



① Pinhead
② Groove

3. Open the Lid using the Open/Close Handle.



① Open/Close Handle
② Stainless Steel Plate

Set the operating angle of the Primary Magnet

Set the incline and position of the Primary Magnet with the Angle Gauge and the Magnet Fixing Pin as illustrated in the following procedure.

IMPORTANT! **Do not turn** the Primary Magnet when the fixing pin is set.

Do not turn the Primary Magnet when the secondary magnet protective cap is used. The magnet grips may hit the secondary magnet.
Do not turn the Primary Magnet when the lid is open. The pin head may hit the Rotation Unit and damage the lid. Make sure the lid is closed.

---

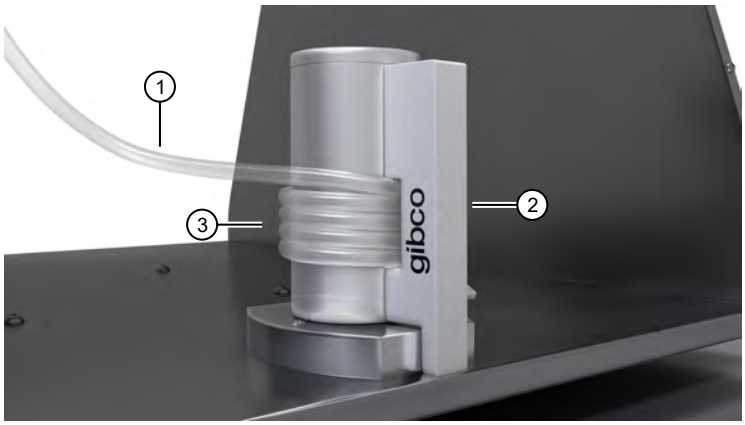
1. Release the Magnet Fixing Pin.

2. Tilt the Primary Magnet to the desired setting on the Angle Gauge.

3. Fix the Magnet Fixing Pin to lock the Primary Magnet in place.

Set up the Secondary Magnet

1. Detach the Secondary Magnet from the base.
2. Wrap the outlet tubing for the fluid downstream of the Primary Magnet around the Secondary Magnet four times.



① Outlet tubing
② Tube Gripper
③ Wrapped tubing

3. Attach the Tube Gripper to hold the tubing in place.
4. Re-attach the Secondary Magnet to the base.

Overview of immunomagnetic separation (IMS)

The CTS™ DynaMag™ Magnet isolates cells bound to Dynabeads™ magnetic beads. Dynabeads™ magnetic beads are superparamagnetic, thus they become magnetized when exposed to a magnetic field and pull towards the magnet. Once removed from the magnetic field, Dynabeads™ magnetic beads have no magnetic remanence, and will resuspend easily when agitated.

Cell isolation

Dynabeads™ magnetic beads are coated with antibodies to bind specific target cells. This allows capture and isolation of target cells with the CTS™ DynaMag™ Magnet whereby unwanted cells are washed away.

Note: *Cell isolation and activation in one step can be achieved with CTS™ Dynabeads™ CD3/CD28 (see the CTS™ Dynabeads™ CD3/CD28 User Guide for details).*

Cell depletion

Depletion can be performed with Dynabeads™ magnetic beads coated with specific antibodies to allow depletion of unwanted cells. While unwanted cells are bound by Dynabeads™ and captured on the CTS™ DynaMag™ Magnet, highly pure target cells can be collected in a separate bag.

Overview of T cell isolation and activation with CTS™ Dynabeads™ CD3/CD28

The following images are a graphical representation of the principle behind T cell isolation and activation with CTS™ Dynabeads™ CD3/CD28 and the CTS™ DynaMag™ Magnet.

CTS™ Dynabeads™ CD3/CD28 bind to target T cells, while non-T cells remain unbound in solution.

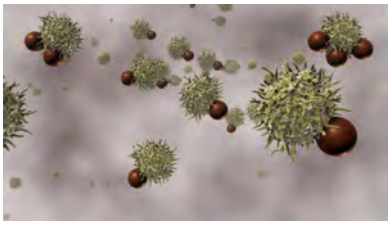


Figure 5 T cell binding

When placed on the CTS™ DynaMag™ Magnet, Dynabeads™-bound target T cells are pulled towards the magnet will be pulled towards the magnet.

Figure 6 Magnetic attraction of T cells

Dynabeads™-bound target T cells are captured on the magnet while unbound cells can be removed, leaving a pure, viable T cell population behind.

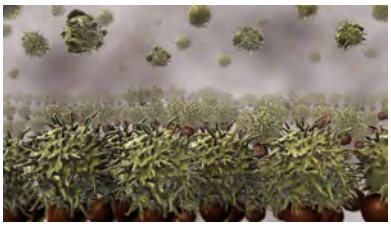


Figure 7 T cell capture and isolation

The combination of CD3 and CD28 antibodies on the surface of CTS™ Dynabeads™ CD3/CD28 provides both the primary and co-stimulatory signals that are required for optimal activation and expansion of T cells.

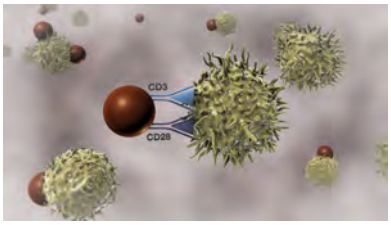


Figure 8 T cell activation and expansion

Cell isolation with the CTS™ DynaMag™ Magnet and Dynabeads™ magnetic beads

The procedure for pre-treating cell suspensions varies depending on the specific Dynabeads™ product used. The operator is recommended to use the Instruction for Use for the specific CTS™ Dynabeads™ products. If another Dynabeads™ product is used we recommend using the same conditions e.g., time, temperature, concentrations for incubation of the cells with the Dynabeads™ magnetic beads as in the Instruction for Use as a starting point.

For clinical applications a closed single use bag system is recommended for cell isolation and washing procedures in combination with the CTS™ DynaMag™ Magnet.

Cell depletion with the CTS™ DynaMag™ Magnet and Dynabeads™ magnetic beads

Cells can be depleted from a complex cell population by binding of specific unwanted cells by CTS™ Dynabeads™, subsequent capture with the CTS™ DynaMag™ and draining of a clean and Dynabeads™ free cell population in a separate bag. The process for cell depletion may be optimized for individual processes.

Single use consumables for T cell isolation and depletion

Use aseptic techniques such as sterile spike connectors or sterile welding for all procedures. A representation of a closed bag system is shown in Figure 9. Bag 1 (isolation bag), containing the bead-cell suspension is connected to bag 2 (wash buffer) and bag 3 (cell culture media).

Note: *Isolation can be performed in an ordinary consumable bag or in a gas-permeable bag for direct cell culture after isolation.*

Figure 9 Consumable kit (assembly) for cell isolation and subsequent addition of cell culture media.

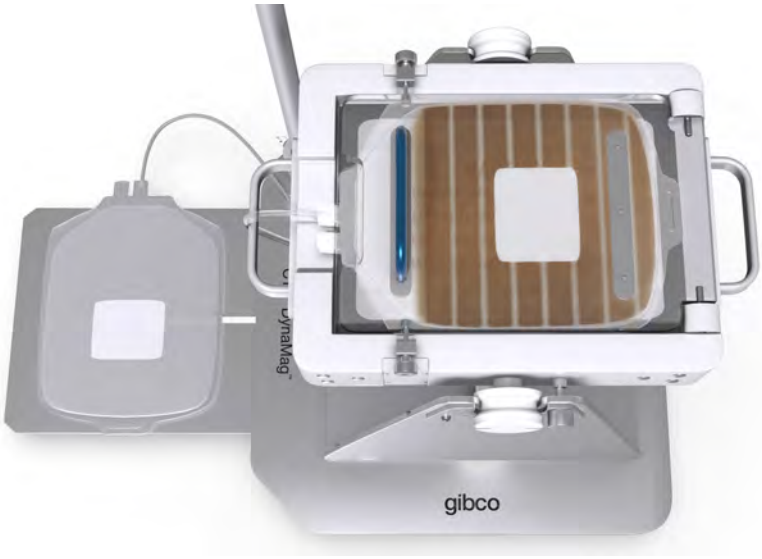
① Isolation Bag
② Wash Buffer
③ Cell Culture Bag
④ Tube A
⑤ Tube B

Bind T cells with CTS Dynabeads CD3/CD28

1. Prepare CTS™ Dynabeads™ CD3/CD28 and your cell starting material according to the specific instructions in the user guide (MAN0008945).
2. Aseptically add the target cell suspension and the required amount of washed Dynabeads™ magnetic beads to bag 1.
3. Place bag 1 on a sample mixer and mix at 1-3 rpm for 30 minutes at room temperature to gently mix the cells and CTS™ Dynabeads™ cell complexes.

Capture of Dynabeads™-bound T cells with the CTS™ DynaMag™ Magnet

1. Connect bag 2 containing wash buffer and bag 3 containing complete cell culture media to bag 1 for cell isolation. See the *CTS™ Dynabeads™ CD3/CD28 User Guide* (MAN0008945) for recommended volumes.
2. Ensure that all tubing line clamps on the connection lines are closed.
3. Hang bag 2 with the wash buffer on the Solution pole.
4. Add wash buffer from bag 2 to bag 1 by opening the line clamp on tube B. Use approximately three times the volume that was used for isolation.
5. Close the line clamp on tube B.
6. Tilt the magnet to a position of -15°.
7. Open the lid of the Primary Magnet and gently remix the contents of bag 1.
8. Place bag 1 on the Primary Magnet with the bag ports towards the raised side of the magnet.



9. Close the lid and incubate for 1 minute. The lid compresses the bag and ensures that Dynabeads™ magnetic beads are kept within optimal distance of the Primary Magnet.
10. Pull out the retractable plate and place the now empty bag 2 on the plate.
11. Hang bag 3 with the complete cell culture medium on the Solution pole with the line clamp closed.

12. Carefully drain the non-target cells from bag 1 to the now empty bag 2 by releasing the line clamp on tube B and by inclining the Primary Magnet up to 60°.

    Note: The angle of the Primary Magnet influences the flow rate and can be optimized for individual processes.

13. Immediately remove bag 1 containing the Dynabeads-captured cells from the Primary Magnet and place it on the retractable plate.

14. Open the line clamp on tube A to add complete cell culture medium from bag 3 to bag 1.

15. Gently resuspend the cell/bead complexes.

16. Positively isolated bead-bound cells are now ready for downstream applications.

    Note: If a gas permeable bag is used for isolation, bag 1 can be placed directly in an incubator at 37°C/5% CO2. If a non-gas-permeable bag was used for isolation, transfer the media containing T cell/CTS™ Dynabeads™ CD3/CD28 complexes from bag 1 to a suitable cell culture flask or bioreactor. Wash bag 1 with additional cell culture medium and transfer the media to the same cell culture flask or bioreactor.

17. Allow isolated and activated cells to remain undisturbed until day 3 of culture.

18. Collect a sample of the non-captured cell fraction and assess isolation efficiency and cell recovery with help of CD3+ T cell numbers in the starting fraction and in the non-captured fraction. See “Determine isolation efficiency and cell recovery”.

Determine isolation efficiency and cell recovery

Cell isolation efficiency and cell recovery can be determined indirectly by assessing the number of CD3+ T cells present in bag 3 after T cell isolation.

1. Prior to isolation, collect a sample from the starting fraction (same cell concentration as bag 1) and store it in a tube.

2. After draining of the unbound cell fraction from bag 1 and repeated addition of washing volumes from bag 2 to bag 3, collected a sample from bag 3 (see the previous section for details).

3. Dilute the sample from the starting fraction to match the addition of washing volumes from bag 2 (eg. if the volume in bag 3 is increased due to wash steps dilute the starting sample accordingly).

4. Stain both samples with a fluorochrome conjugated anti-CD3 antibody (optional: include anti-CD28 antibody to evaluate double positive CD3CD28 isolation efficiency). Prior to flow analysis add a volumetric control (bead counting standard) to both samples in order to harvest the same volume of each sample for flow cytometric analysis.

5. Calculate the amount of target cells that was isolated from the starting fraction by the following formula:

$$\text{Cell recovery} = 1 - \left(\frac{\text{Target cell events from unbound cell fraction}}{\text{Target cell events from starting fraction}} \right) x\ 100\%$$

Overview of Dynabeads™ removal with the CTS™ DynaMag™ Magnet

The following images are a graphical representation of the principle behind Dynabeads™ removal with the CTS™ DynaMag™ Magnet.

CTS™ Dynabeads™ CD3/CD28 naturally detach from T cells after 4-5 days of cell expansion.

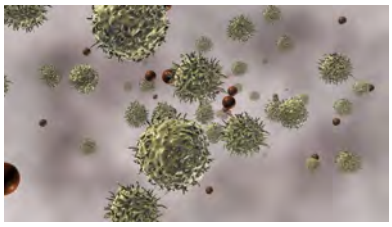


Figure 10 CTS™ Dynabeads™ CD3/CD28 detachment

When placed on the CTS™ DynaMag™ Magnet, Dynabeads™ will be pulled towards the magnet.

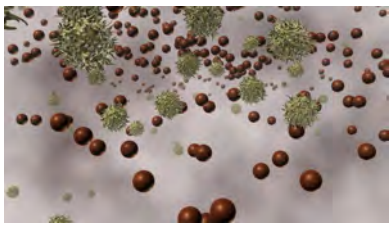


Figure 11 Dynabeads™ magnetic attraction

Dynabeads™ are immobilized on the CTS™ DynaMag™ Magnet.

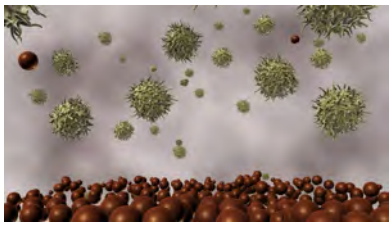


Figure 12 Dynabeads™ immobilization

A Dynabeads™-free, highly pure population of expanded T cells can be collected in a separate bag.

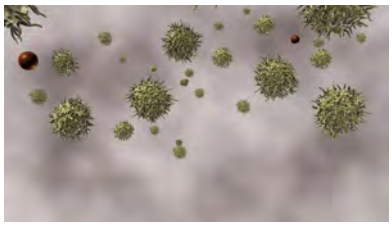


Figure 13 Dynabeads™-free expanded T cells

Removal of CTS™ Dynabeads™ with the CTS™ DynaMag™ Magnet

The CTS™ DynaMag™ Magnet is used to remove Dynabeads™ magnetic beads from an isolated target cell population. Cells activated with CTS™ Dynabeads™ CD3/CD28 or CTS™ Dynabeads™ Treg Xpander are recommended to be removed after 4–5 days of culture to minimize cell loss (see the corresponding product manuals for details).

About single use consumables for Dynabeads™ removal

Use aseptic techniques such as sterile spike connectors or sterile welding for all procedures. A representation of a closed bag system for removal of Dynabeads™ after cell expansion is shown in Figure 14. Bag 1, containing the expanded cells is connected to bag 2 (wash buffer) and an empty bag 3.

Note: Dynabeads™ removal can be performed as static process or by flow-through with help of a peristaltic pump to ensure the flow of a larger volume over the Primary Magnet and Secondary Magnet in a controlled manner.

Figure 14 Consumables kit for Dynabeads™ removal.

① Expanded T cells
② Wash Buffer
③ Empty Bag
④ Tube A
⑤ Tube B

Remove CTS™ Dynabeads™ CD3/CD28 with the CTS™ DynaMag™ Magnet

1. For Dynabeads™ removal in a static process, place bag 1 on the Primary Magnet and ensure that the lid closes for optimal bead removal performance. Air can be removed aseptically with help of a syringe is necessary.

   Note: The lid closes with a max. volume of 330 mL in a 1-L consumable bag. For volumes larger than 330 mL the volume must be either separated in several bags or a flow-through process needs to be used.
   Smaller bags can be used if the total filling volume allows the lid to close.

2. Aseptically connect the bag with expanded cells (bag 1) to bag 2 containing wash buffer and an empty bag 3 for collection of Dynabeads™-free cells.

3. Ensure that all tubing line clamps on the connection lines are closed.

4. Hang bag 2 with the wash buffer on the Solution Pole.

5. Tilt the Primary Magnet to a position of -15 degree and install bag 1.

6. Close the lid. The lid compresses the bag and ensures that Dynabeads™ magnetic beads are kept within optimal distance of the Primary Magnet.

7. Backflush a small volume of wash buffer from bag 2 into bag 1 by opening the line clamps on tube B. Close the line clamp again. This flushes Dynabeads™ magnetic beads (which would otherwise escape magnetic capture) from the outlet port, and into bag 1.

8. Detach the Secondary Magnet from its base. Wrap the outlet tubing (tube A) around the pillar four times (See “Set up the Secondary Magnet” on page 15) and add the Tube Gripper to hold the tubing in place. Re-attach the Secondary Magnet to its base.

9. Pull out the retractable plate and place the empty collection bag (bag 3) on the plate.

10. Carefully drain the bead-free fraction from bag 1 by releasing the line clamp of tube A. Ensure that the line clamp of the wash buffer (tube B) is closed. The Primary Magnet can be tilted up to 60° during this step (See “Set the operating angle of the Primary Magnet” on page 14).

    Note: When using a peristaltic pump, a flow rate of 20–30 mL/min is recommended for optimal magnetic capture efficiency. Faster flow rates might result in beads escaping the magnetic capture into the collection bag. In addition, a horizontal or negative magnet angle is recommended.

11. When bag 1 is almost empty, stop the flow with the line clamp. Do not allow the bag to empty. The efficiency of the magnetic capture of the Dynabeads™ magnetic beads is reduced if air bubbles enter the tubing.

12. Open the Primary Magnet lid and remove bag 1. Open the line clamps on tube B and drain wash buffer into bag 1. Gently resuspend the bead-cell complexes.

13. Repeat steps 3–12 twice. The number of washing steps may be optimized.

14. Bead-free cells are now ready for downstream applications.

Determine residual beads in your cell population

The following procedure can be used to determine the number of residual beads:

Note: Alternative protocols can be found in *Levine B.L., et. al. J Hematotherapy, 7:437–488. (1998) and Hollyman et. al., J Immunother, Vol 32, No 2:169–180 (2009)*.

1. Transfer a sample of 3×10^6 viable CD3+cells from the released cell fraction to a microcentrifuge tube.

2. Centrifuge at max speed in a table-top centrifuge for 2 minutes and remove the supernatant.

3. Add 0.5 mL RIPA Lysis and Extraction Buffer (Cat. No. 89901) buffer and resuspend without generating bubbles.

4. Add 10 µL DNase I (Cat. No. 18047019) and resuspend carefully using a P1000 pipette.

5. Incubate at 37°C for 20 minutes.

6. Add 50 µL Proteinase K Solution (Cat. No. 125530049) and resuspend carefully using a P1000 pipette.

7. Incubate at 65°C for 20 minutes.

8. Centrifuge at max speed for 2 minutes and carefully remove the supernatant.

9. Add 7 µL RIPA buffer (expect ≈2 µL residual volume, resulting in total volume 9 µL).

10. Vortex briefly.

11. Load the entire sample onto on a KOVA Glasstic microscope slide (Kova # 22-270141).

12. Record the number of CTS™ Dynabeads™ CD3/CD28 beads according to the KOVA Glasstic manual.

3 Optional applications

The Primary Magnet can be inclined stepwise to optimize separation (Figure 3 on page 8). Additionally, optional configurations allow the magnet to be positioned below or above the sample bag (Figure 15 on page 27). When the magnet is positioned above the sample bag, the bead-cell complexes have to move against gravity, thus avoiding unspecific cell trapping.

Change the configuration of the Primary Magnet

To optimize magnetic separation of rare cell populations below 3% of the total cell population, the CTS™ DynaMag™ system offers an alternative configuration with the Primary Magnet placed above the blood bag. In this position, the bead-cell complexes are driven against gravity by magnetic forces, avoiding unspecific cell trapping. Note: the lid shutting pin must be secured before starting this process.

1. Remove Secondary Magnet.
2. Release the Magnet Fixing Pin, then flip the Primary Magnet by 90° to a vertical position.
3. Pull up the Locking Pin to release the Rotation Unit.
4. Turn the Rotation Unit 180°.
5. The Locking Pin automatically locks the bases after the Rotation Unit is fully turned.
6. Flip the Primary Magnet forward 90° to a horizontal position.
7. Push in the Magnet Fixing Pin to lock the Primary Magnet.
8. Replace the Secondary Magnet.

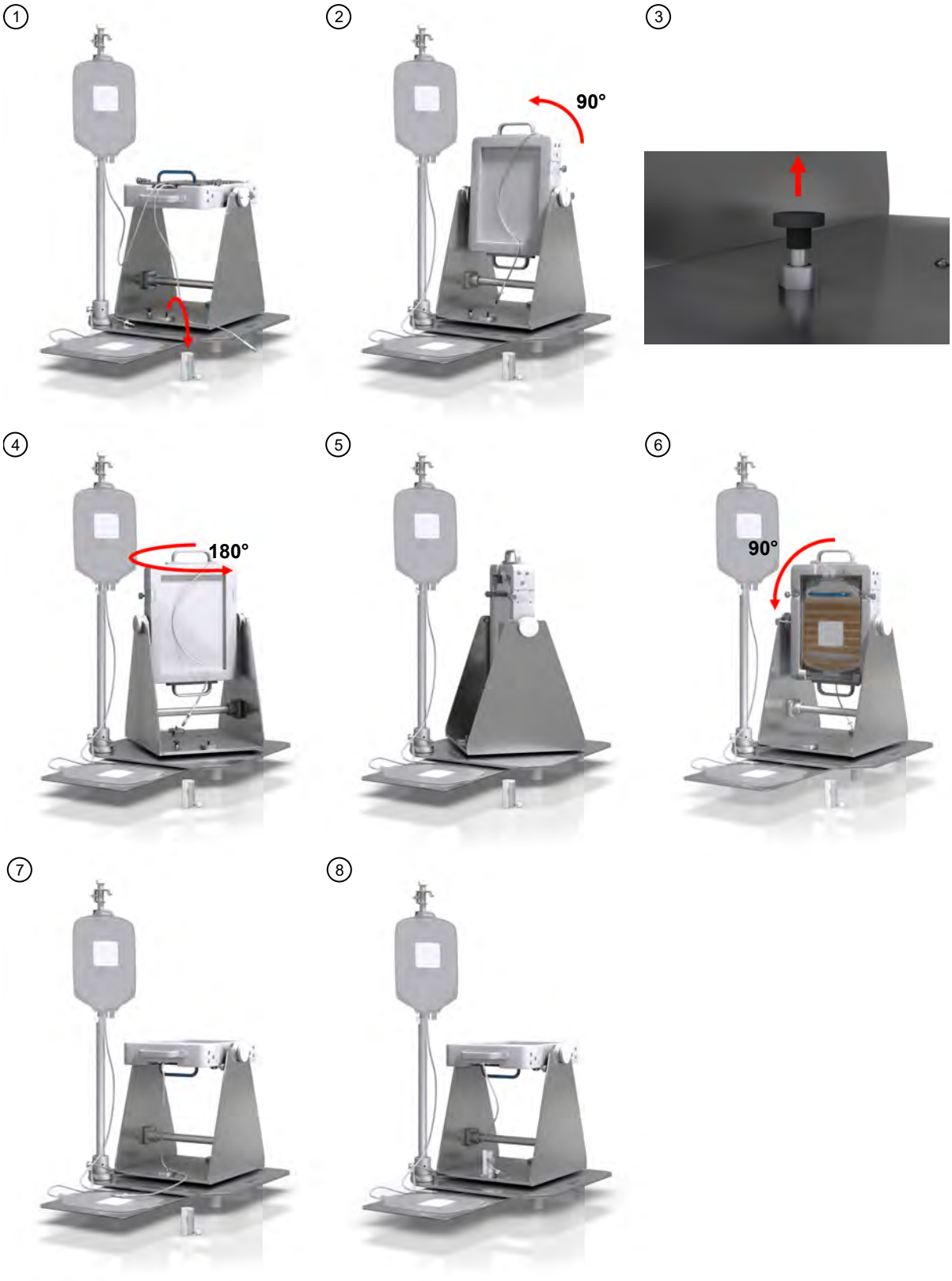


Figure 15 Changing the configuration of the Primary Magnet

4 Maintenance

Cleaning and maintenance of the magnet

CAUTION! Fix the Primary Magnet to the base with the Magnet Fixing Pin when washing. Unexpected rotation of the magnet may cause injury.

- Treat biological materials as potentially infectious and wear appropriate protective clothing during cleanup procedures.
- Disinfect surfaces that have been in contact with blood or blood components with with 70% GMP isopropyl alcohol wipes.
- Avoid chemical disinfectants containing chloride or acids as they may cause corrosion.
- Do not immerse in fluids and avoid prolonged exposure to aqueous solutions.
- Do not autoclave or use abrasive or strong solvent cleaners.
- Avoid exposure to temperatures above 50°C, strong magnetic fields, UV light, direct sunlight, and vibrations that may damage the sheathing.
- The magnetic field is stable and measurement of the magnetic strength by the customer is not recommended as the in-situ measurement with tesla meter /gauss meter is not suitable and may provide incorrect measurement results. The sheeting can influence the measurement of the magnetic field. Measurement can therefore only be carried out without sheathing by the manufacturer.

Certificate of Conformity

A Certificate of Conformity is supplied with each device, and provides detailed quality control and product qualification information for each product.

Serial number

For your own reference, record the serial number of your CTS™ DynaMag™ Magnet in the space provided. The serial number can be found on the machine label on the base plate of the unit.

Serial Number _____

Date Received_____

A

Freqently asked questions

Frequently asked questions

Question	Answer
Does Thermo Fisher Scientific offer IQ, OQ?	We do not offer IQ, OQ for the CTS™ DynaMag™ Magnet as it is a mechanical device. A video on how to operate CTS™ DynaMag™ Magnet is provided on the product web page.
Is calibration needed for the CTS™ DynaMag™ Magnet?	The magnetic field is stable when stored at ambient temperature and measurement of the magnetic strength by the customer is not recommended as the in-situ measurement with tesla meter /gauss meter is not suitable and may provide incorrect measurement results. The sheeting can influence the measurement of the magnetic field. Measurement can therefore only be carried out without sheathing by the manufacturer. Each CTS™ DynaMag™ Magnet has a unique serial number and is delivered with a Certificate of Conformity stating that the unit is manufactured in compliance with approved specifications and ISO 9001 regulatory requirements.
Why is the magnetic strength not even on the Primary Magnet?	The Primary Magnet consists of an array of extremely strong permanent Neodymium-Iron-Boron magnets. The arrangement is optimized to give high field strength and a favorable field gradient, which ensures efficient separation of Dynabeads™ M-450 magnetic beads. Due to the arrays of magnets is the magnetic field not even throughout the Primary Magnet.
What single use sterile bags can I use on the CTS™ DynaMag™ Magnet?	The Primary Magnet has measures 170 mm (width) × 200 mm (depth). Sterile single use bags within this measure can be used. The lid allows a distance of 11 mm or less from the Primary Magnet surface, which is possible with sample volumes up to 330 mL in 1000 mL bags in static separations.
How should I store the CTS™ DynaMag™ Magnet?	We recommend storing it at ambient temperature and avoid temperatures above 50°C, strong magnetic fields, UV light, direct sunlight, vibration that may change the sheeting.

(continued)

Question	Answer
How many residual Dynabeads™ magnetic beads are allowed in the final product?	Thermo Fisher Scientific has not conducted any *in vivo* toxicological studies with Dynabeads™ magnetic beads, however summarized below, are studies and data conducted/generated by our customers using our Dynabeads products: The potential for acute toxicity from Dynabeads™ M-450 Sheep Anti-Mouse IgG ST (SAM-Beads) administered once intravenously to male and female rats was assessed [1]. Rats administered 9.6×10^4 beads/kg were killed 14 days post-treatment. Rats administered 8.3×10^8 beads/kg were killed either 14 or 42 days post-treatment. Saline containing 0.5% PPF served as the control article. Treatment groups were statistically compared with respect to clinical chemistry, haematology parameters, and body weight data. No significant group differences were detected ($\alpha = 0.01$) with respect to any statistically analyzed data. No test beads were found in any of the tissues from animals administered the lowest dose 9.6×10^4 beads/kg. For animals administered the highest dose 8.3×10^8 beads/kg the majority of the SAM-Beads were found in the lung, liver, and spleen and were slightly more numerous among animals who were killed at 14 days. There was also a trend toward an increased incidence and/or distribution of phagocytized beads in the bone marrow of animals killed 42 days posttreatment when compared with the 14-day killed animals. A few extracellular beads were present in the lymph nodes, kidneys, and sternal bone marrow. Under the conditions of this study, intravenous administration of Dynabeads™ M-450 Sheep Anti-Mouse IgG ST did not result in any adverse test-article-related macroscopic, clinical pathologic, or histopathologic changes. • The lowest dose in [1] was 9.6×10^4 beads/kg and equals for a 50 kg person 4.8×10^6 beads. • The commonly used criteria in the market by our customers is <100 beads/3×10^6 cells [2]. This is based on the results in the toxicity study [1]. 1. White RD, Glosson JA, Gordon DE, et al., Intravenous Safety Study in Rats Given Paramagnetic, Polystyrene Beads with Covalently Bound Sheep Anti-Mouse Immunoglobulin G (IgG). International Journal of Toxicology. Vol 14, Issue 4, pp. 251–265 2. Hollyman D, Stefanski J, Przybylowski M. Manufacturing Validation of Biologically Functional T Cells Targeted to CD19 Antigen for Autologous Adoptive Cell Therapy. J Immunother 2009_ Volume 32, Number 2, pp. 169–180 (Table 5 Release Testing Criteria and Results on the EOP 1928z-transduced T Cells)

(continued)

Question	Answer
The Certificate of Conformity (CoC)supplied with the CTS™ DynaMag™ Magnet has been lost. Where can a copy of the CoC be obtained?	A copy of the CoC will be provided upon request. Please contact techsupport@thermofisher.com and provide the serial number of your device. The serial number can be found on the machine label on the base plate of the unit.
How does the angle of the Primary Magnet influence the flow rate during Dynabeads™ magnetic bead removal?	A steep angle of the Primary Magnet, a large tube diameter and the height of filled bags increase the flow rate during a static Dynabeads™ magnetic bead removal process. The angle of the magnet may be optimized for individual processes. When performing Dynabeads™ magnetic bead removal with a peristaltic pump a horizontal position of the Primary Magnet and a flow rate between 20–30 mL/min are recommended. Dynabeads™ magnetic beads that escape capture by the Primary Magnet are captured in the tubing that is wrapped around the secondary magnet.
Do Dynabeads™ magnetic beads and target cells form aggregates after the isolation process?	Yes, this is normal. During isolation, cells and Dynabeads™ magnetic beads form rosettes, which resemble small aggregates. This is a normal and necessary phenomenon for successful cell activation and proliferation. We recommended to leave cell aggregates undisturbed for 2–3 days to allow optimal cell activation. Aggregates might enlarge due to natural cell proliferation and might also form later during the expansion process.
Can I use smaller bags for isolation and bead removal together with the CTS™ DynaMag™ Magnet?	Yes, any bag size that fits within the frame of the Primary magnet can be used. The maximum filling volume for individual bags should be determined to allow the lid to be closed during the process.

B

Specifications

Technical specifications

Feature	Specification
Catalog Number	12102
Serial Number	Specific for each device
Intended Use	For Research Use Only or Manufacturing of Cell, Gene or Tissue Based Products
Storage	Ambient temperature
Overall Dimensions	430 mm (width) × 390 mmm (depth) × 500 mm (height with pole retracted) 740 mm (height with pole extended)
Overall Weight	27 kg
Rotation – horizontal	0°, 90° and 180°
Regulatory requirements	ISO 9001
Hardware functional test	Passed
Primary Magnet	
Overall dimensions	326 mm (width) × 404 mm (depth) × 64 mm (height)
Weight	14 kg
Magnet dimensions	170mm (width) × 200 mm (depth)
Magnetic strength	>8k Gauss
Extendable solution pole mounted on the base unit	500 mm to 740 mm (height)
Rotation – vertically	-15°, 0°, 15°, 30°, 45°, 60°, 90°, 165°, 180°, and 195°
Maximum bag size	190 mm (width) × 235 mm (depth)
Secondary Magnet	
Magnet dimensions	38.1 mm (diameter) × 75 mm (height)
Protective Cap dimensions	56 mm (diameter) × 90 mm (height)

Warning and limitations

This product guarantees optimum isolation of Dynabeads™ magnetic beads, not the isolation of a specific material. Recovery of bio-molecules by magnetic isolation depends on the avidity of the antibodies or ligands on the surface of Dynabeads™ magnetic beads, as well as factors concerning the biomolecules themselves and the matrix from which they are to be isolated.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.

Safety instructions for the CTS™ DynaMag™ Magnet

The CTS™ DynaMag™ Magnet comprises two very strong permanent magnets for separation of paramagnetic Dynabeads™ magnetic beads with or without bound cells.

The magnetic strength of the Primary Magnet is 8,000 Gauss (0.8 T) while the Secondary magnet is 2,500 Gauss (0.25 T).

Carriers of medical devices or medical implants are strongly advised to not transport the CTS™ DynaMag™ Magnet, assemble or use the device without prior medical consultation in order to avoid possible health hazards.

EHS professionals should be consulted to further inspect the entire working area for possible risks and to provide additional safety instructions, if necessary. Optionally, third party providers like workplace safety consultants could be enlisted. Please note, that the magnetic field strength decreases exponentially with distance. However, the angle of the primary magnet, as well as the position of the magnet on the laboratory bench influence the actual magnetic field exposure which is why the actual exposure needs to be assessed for each situation individually.

No ferromagnetic materials or materials that are sensitive to magnetic fields should come in close vicinity of the magnets.

We recommend including these safety instructions in the training for all users of the CTS™ DynaMag™ Magnet as well as any laboratory or clean room personnel.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

D

Ordering information

Accessory products

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Product	Amount	Cat. No.
CTS™ Dynabeads™ CD3/CD28	10 mL	40203D
Dynabeads™ Human T-Expander CD3/CD28	10 mL	11141D
CTS™ Dynabeads™ Treg Xpander	10 mL	46000D
CTS™ DPBS without calcium chloride, without magnesium chloride	1 L	A1285601
Labtainer™ BioProcess Container (BPC), 250mL with 2 Ports, Luer Lock, 2D BPC	10/case	SH30658.13
Labtainer™ BioProcess Container (BPC), 500mL with 2 Ports, Luer Lock, 2D BPC	10/case	SH30657.14
RIPA Lysis and Extraction Buffer	250 mL	89901
DNase I	20,000 units	18047-019
Proteinase K Solution (20 mg/mL), RNA grade	5 mL	25530-049
CTS™ OpTmizer™ T-Cell Expansion SFM	1 L (bottle) 1 L (bag)	A1048501 A1048503
CTS™ OpTmizer™ T-Cell Expansion SFM, no phenol red	1 L (bottle) 1 L (bag)	A3705001 A3705003
CTS™ OpTmizer™ Pro Serum Free Medium (SFM), No Phenol Red	1 L (bottle) 1 L (bag)	A4966101 A4966103
CTS™AIM-V™ Medium	1 L	0870112DK
CTS™ Immune Cell SR	50 mL 500 mL	A25961-01 A25961-02

(continued)

Product	Amount	Cat. No.
CTS™ IL-2 Recombinant Human	100 µg 1 mg	CTP0021 CTP0023
Digital Rocker, 120V US/JP plug	Pack of 1	88882001
Digital Rocker, 230V EU/UK/CHN Plug	Pack of 1	88882002

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.