BD Horizon™ Dri Tumor & Tissue Dissociation Reagent

Catalog No. 661563 **15 Tests/kit**

RESEARCH APPLICATIONS

- Dissociation of solid tumor biopsies into single cell suspension (optimized for breast cancer)
- Sample preparation for downstream single-cell analyses such as flow cytometry/cell sorting and genomics applications (next-generation sequencing [NGS], RNAseq)

PRODUCT DESCRIPTION

The BD Horizon™ Dri Tumor & Tissue Dissociation Reagent (TTDR) is designed to enable solid tumor tissue disaggregation into a single-cell suspension from fresh or overnight stored or shipped xenografts and primary tumors from breast cancer specimens. The reagent has not been tested for formalin-fixed paraffin-embedded (FFPE) tumor tissues. The dissociation reagent has been optimized for viable cell yield of both tumor cells and immune infiltrates without any selection bias. This reagent has been formulated for better preservation of commonly used cell surface markers on tumor and infiltrating immune cells. The resulting single-cell suspension is suitable for downstream analysis to explore tumor heterogeneity and tumor microenvironment using flow cytometry, such as isolation of cell subpopulations by cell sorting, followed by molecular analysis such as NGS gene expression analysis.

KIT COMPONENTS

The reagent is provided as 15 single-use amber vials per kit. Each vial contains sufficient reagent to dissociate up to 1.0 g of tissue.

NOTE It is feasible to dissociate tissue weighing outside this range. However, it should be tested by the user.

STORAGE AND HANDLING

Store vials dry at 2°C–8°C. See vial label for expiration date.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[9,10] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

The reagent is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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	Danger
	H315: Causes skin irritation. H319: Causes serious eye irritation. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. H335: May cause respiratory irritation.
Prevention	P264: Wash thoroughly after handling. P280: Wear protective gloves/protective clothing/eye protection/face protection. P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P284: [In case of inadequate ventilation] wear respiratory protection. P271: Use only outdoors or in a well-ventilated area.
Response	P342+P311: If experiencing respiratory symptoms: Call a POISON CENTER/doctor/... P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313: If eye irritation persists: Get medical advice/attention. P302+P352: IF ON SKIN: Wash with plenty of water/... P332+P313: If skin irritation occurs: Get medical advice/attention. P312: Call a POISON CENTER/doctor if you feel unwell. P321: Specific treatment (see Safety Data Sheet). P362: Take off contaminated clothing.
Storage	P403: Store in a well-ventilated place. P233: Keep container tightly closed. P405: Store locked up.
Disposal	P501: Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

Go to regdocs.bd.com to download the Safety Data Sheet.

PROCEDURE

Additional reagents and materials required but not provided

- Dulbecco's phosphate buffer saline (DPBS) without Ca^{++}/Mg^{++}
- BD Pharm Lyse™ Lysing Buffer (Catalog No. 555899)
- Dulbecco's modified Eagle medium (DMEM)
- 1% BSA/DPBS/2 mM EDTA (without Ca^{++}/Mg^{++})
- (Optional) BD FACS™ Pre-Sort Buffer (Catalog No. 563503)
- 50-mL conical centrifuge tubes
- 70-µm cell strainer
- Scalpel or other means of mincing tissue
- Glass Petri dish, 100×20-mm

Preparing 2X TTDR

To make 2X TTDR solution:

1. Add 5 mL of DMEM to the amber vial containing TTDR.

2. Gently agitate periodically for 15 minutes at room temperature to ensure complete reconstitution of the dried reagent.
3. Transfer the reconstituted TTDR to a labeled 50-mL conical centrifuge tube. Discard amber vial.
4. Store at 4°C until needed.

   Use the reconstituted TTDR within 24 hours to ensure maximal activity.

Preparing the tissue

To prepare the tissue specimen:

1. Place the tissue specimen on ice.
2. Weigh the tissue specimen and place the tissue into a fresh, labeled 100 × 20-mm glass Petri dish containing 5 mL of 37°C DMEM.

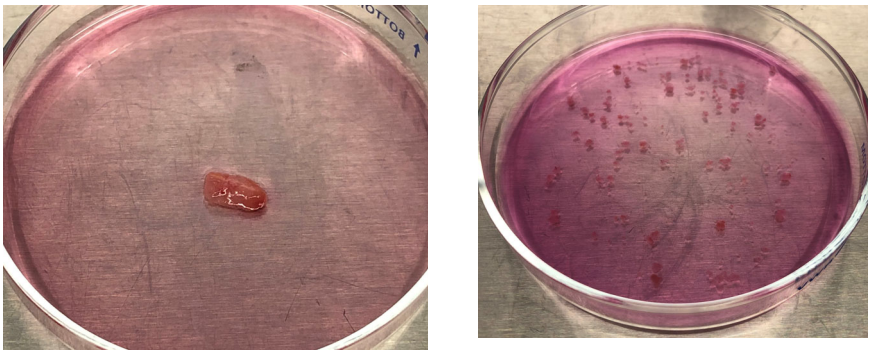
   NOTE Based on the weight of the tumor, determine how much TTDR to use. It is recommended that 1 vial of reconstituted dissociation reagent be used for no more than 1 g of tissue. If your sample is larger than 1 g, it might be necessary to divide the specimen into multiple pieces and process them separately using 1 vial of reconstituted TTDR for each piece.

Mincing the tissue

1. Use two scalpels, or other method of choice, to finely mince the tissue in the Petri dish.

   The resulting tissue pieces should be as small as possible, with no pieces larger than 0.75 mm in diameter (see Figure 1). Inadequate mincing can significantly reduce cell yield.

Figure 1 Tumor before and after mincing



2. Approximately 30 minutes before tissue mincing has been completed, place the tube containing 2X TTDR in a 37°C water bath.

   NOTE Do not leave the TTDR in the 37°C water bath for longer than 30 minutes prior to use.
3. Transfer contents of the Petri dish (DMEM and tumor pieces) into the 50-mL conical tube containing 37°C 2X TTDR.

   The final volume in the 50-mL conical tube should be 10 mL (5 mL warm TTDR + 5 mL minced tumor in DMEM).

   NOTE If processing multiple dissociations, mince the tissue in the first Petri dish and transfer the contents to a fresh, labeled 50-mL conical tube. Place tubes on ice until all of the tissue pieces have been minced. After all of the tissue pieces have been minced, add 5 mL of 2X TTDR to each tumor preparation to ensure simultaneous incubation start times.

Digesting the minced tissue

1. Incubate the tube(s) containing the minced tissue and TTDR at 37°C for 30 minutes with mild but frequent agitation.

This can be achieved using a shaking water bath or by placing a rocker inside a 37°C incubator. Alternatively, place the digestions in a rack inside the incubator and manually agitate the tubes by gentle inversion every 7-10 minutes.

NOTE Longer or shorter incubation times might result in changes in viability or in changes in the phenotypic distribution of cells in the cell suspension. You can test different incubation times to meet the specific needs of the experiment.

2. After incubation, add 25 mL of 1% BSA/DPBS/2 mM EDTA to the 50-mL conical centrifuge tube(s) containing the dissociated tissue to bring total volume to 35 mL.
3. Pass the contents of each tube through a fresh 70-µm cell strainer into a fresh, labeled 50-mL conical tube.
4. Centrifuge the tubes at 250*g* for 8 minutes at room temperature.
5. Remove the supernatant and resuspend pellets in 2 mL of 1X BD Pharm Lyse™ solution.
6. Incubate for 15 minutes at room temperature.
7. Add 40 mL 1% BSA/DPBS/2 mM EDTA.
8. Centrifuge at 250*g* for 8 minutes at room temperature.
9. Remove the supernatant and resuspend pellets in 2 mL DPBS/2 mM EDTA.

   NOTE To avoid clumping, use BD FACS™ Pre-Sort Buffer. See the reagent Technical Data Sheet for more information.
10. Count the cells using a hemacytometer or an automated cell counter.
11. Record cell counts and viability.

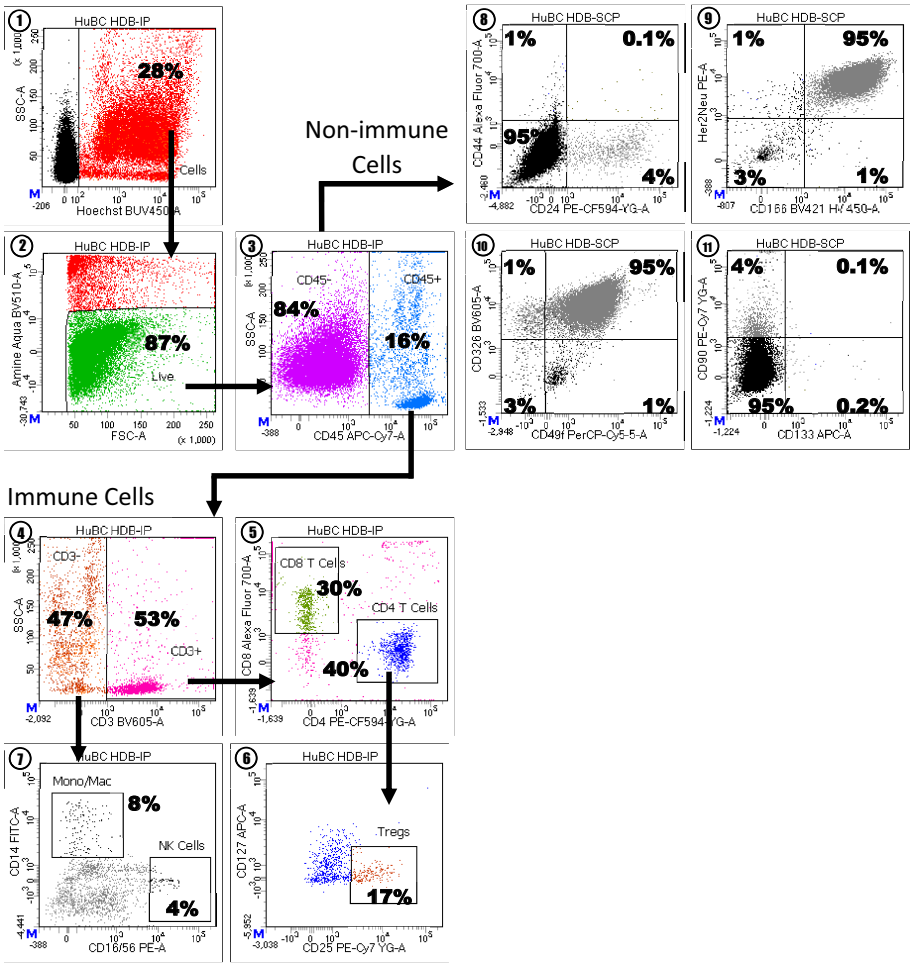
    The cell suspensions are ready to be used for flow cytometry analysis, cell sorting, and other downstream applications.

REPRESENTATIVE DATA

A solid tumor biopsy from a breast cancer patient was dissociated using the BD Horizon™ Dri Tumor & Tissue Dissociation Reagent following the protocol described. The cell suspension was stained for surface markers to identify immune infiltrates ($CD45^+$ cells) and non-immune cells, such as cancer cells ($CD45^-$ cells). The samples were analyzed on a BD LSRFortessa™ flow cytometer with BD FACSDiva™ software using the analysis strategy in the table.

Number	Dot plot(s)	Purpose
1	Hoechst 33342-A vs. SSC-A	Debris is gated out and nucleated cells are identified for further analysis.
2	FSC-A vs. Amine-A	Determine cell viability. Amine-negative events denote live, nucleated cells.
3	CD45 APC-Cy7-A vs. SSC	Distinguish between immune cells ($CD45^+$) and non-immune cells ($CD45^-$)
4–7	Various (immune cells)	Live, nucleated $CD45^+$ immune cells are further analyzed for subset composition.
8–11	Various (non-immune cells)	Live, nucleated $CD45^-$ non-immune cells are further analyzed for expression of known cancer cell markers.

Figure 2 Expression of cell surface markers on dissociated tumor cells



WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

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TRADEMARKS