

# Anti - PD-L1

## Rabbit clonal antibody

### CAT#

#### CONCENTRATED

DB 241-0.1	(100 µl)
DB 241-0.2	(200 µl)
DB 241-0.5	(500 µl)
DB 241-1	(1 ml)

#### READY TO USE (RTU)

DB 241-RTU-7	(7 ml)
DB 241-RTU-15	(15 ml)

### STORAGE AND APPLICATION

#### CONCENTRATED

**Storage:** +4°C  
**Application:** IHC-P,  
 dilution 1:100 - 1:200

#### READY TO USE (RTU)

**Storage:** +4°C, Do not freeze!  
**Application:** IHC-P,  
 ready to use

### PRODUCT INFORMATION

**Clone:** Y19-I  
**Buffer:** 20 mM Tris-HCl, pH 8.0  
**Stabilizer:** 20 mg/ml BSA  
**Preservative:** 0.05% NaN<sub>3</sub>  
**Specificity:** Human  
**Expiration:** 24 months from the shipping date  
**Immunogen:** Peptide derived from internal sequence of human PD-L1

**Cellular localization:** cell membrane  
**Positive control:** placenta and lung tissues  
**Protein accession number:** Q9NZQ7

### IHC-P PROTOCOL – INSTRUCTION MANUAL

1. Deparaffinize the section in 3 changes of xylene, 10 minutes each.
2. Wash the section in 96%, 80% and 70% ethyl alcohol for 10 minutes each.
3. Rinse in distilled water, 2 x 5 minutes.
4. Block the endogenous peroxidase by incubating the tissue in 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for 10 minutes.
5. Wash in distilled water, 2 x 5 minutes.
6. For antigen retrieval: Immerse the slide in Tris-EDTA buffer, pH 9.0\* and incubate in water bath for 20-25 minutes at 96-98°C.
7. Remove the staining to room temperature and let the slide to cool (in Tris-EDTA buffer, pH 9.0) for 20 minutes.
8. Rinse in distilled water, 2 x 5 minutes.
9. Wash in 0.05 M Tris-HCl, pH 7.6 buffer supplemented with 0.2% of Tween-20 or PBS buffer supplemented with 0.2% of Tween-20 (**buffer A**), 2 x 5 minutes.

#### CONCENTRATED:

Incubate the section with primary antibody at the **dilution 1:100 - 1:200** for 1 hour in the closed wet chamber.

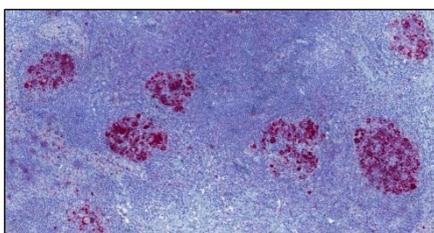
#### READY TO USE (RTU):

Incubate the section with primary antibody (**ready to use**) for 1 hour in a closed wet chamber.

10. Wash 3 x 5 minutes with buffer A.
11. Apply the secondary antibody (the protocol depends on the supplier), and proceed to standard immunohistochemistry protocol (HRP - Peroxide - DAB). Micropolymer-HRP detection kit rabbit/mouse dual of DB Biotech is suggested (<http://www.dbbiotech.com/products/detection-system.html>).
12. Wash 3 x 5 minutes with buffer A.
13. Apply the chromogen (DAB), 1 - 3 minutes.
14. Wash in distilled water, 2 x 5 minutes.
15. Rinse in solution CuSO<sub>4</sub> · 5H<sub>2</sub>O /0.90g NaCl + 0.50g CuSO<sub>4</sub>·5H<sub>2</sub>O in 100ml distilled water/.
16. Wash in distilled water, 1 x 2 minutes.
17. Stain in hematoxylin for 5 minutes.
18. Wash in distilled water, 3 x 2 minutes.
19. Rinse in 37mM ammonium hydroxide solution.
20. Wash in distilled water, 1 x 2 minutes.
21. Mount the slide for observation.

#### \* Tris-EDTA Buffer (10mM Tris Base, 1mM EDTA solution, 0.05% Tween-20, pH 9.0):

Tris ----- 1.21 g; EDTA ----- 0.37 g; Distilled water ----- 1000 ml  
 Mix to dissolve in 700 ml of distilled water. Adjust pH to 9.0 with 1M HCl and then add 0.5 ml of Tween-20 and mix well. Adjust the final volume to 1 liter with distilled water.  
 Store this solution at room temperature for 3 months or at +4°C for longer storage.



Membranous and cytoplasmic positivity of PD-L1 in epithelioid histiocytes and multinucleated giant cells in tuberculous lymphadenitis (alkaline phosphatase chromogen was used in this case).

### VENTANA PROTOCOL – INSTRUCTION MANUAL

#### SHORT APPLICATION PROTOCOL FOR VENTANA BENCHMARK SLIDE STAINING SYSTEM

1. Drying (Enter).
2. Heating glass (72°C), incubation 4 min. Drying.
3. Deparaffinization (Enter).
4. Heating (72°C) with the medium temperatures. Deparaffinization.
5. Prolonged deparaffinization (Enter).
6. Cell conditioning (Enter).
7. ULTRA conditioner #2 (Enter).
8. Heating glass (98°C), incubation 8 min (Cell conditioner #2).
9. ULTRA CC1 solution application – 24 min (Enter).
10. ULTRA CC2 solution application – 36 min (Enter).
11. Titration (Enter).
12. Hand apply – primary antibody. Incubation 28 min.
13. Nuclear stain (Enter).
14. Hematoxylin application – one drop.

### PRECAUTIONS

1. **We strongly recommend to use DB Primary Antibody Diluent (catalog number DB D-125, or DB D-250), eventually alternative diluent (containing protease free BSA at the concentrations ≥ 1mg/ml) for dilution of concentrated antibodies, otherwise the warranty might be voided.**
2. **Centrifuge the vial before use.**
3. Intended for professional In Vitro Diagnostic use in laboratories.
4. Do not use after expiration date stamped on vial label.
5. Avoid contamination of the reagent.
6. Any discrepancies in the recommended procedures stated in the working protocol may affect the final results.
7. The reagent contains sodium azide (NaN<sub>3</sub>) which is highly toxic in higher concentrations. The concentration in the reagent (0.05%) is not considered as hazardous.
8. Disposal of waste material must be conducted in accordance with local regulations.
9. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.