DRG® UBC ELISA (Urinary Bladder Cancer) (EIA-2355)

CE

RUO in the USA

Revised 10 Dec. 1010 rm (Vers. 1.1)

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

The UBC ELISA is a one step assay for determination of cytokeratin 8 and 18 in urine.

2 PRINCIPLE OF THE ASSAY

UBC ELISA is a solid phase sandwich assay based on immunochemical technique. Standards, controls and samples react simultaneously with solid phase catcher antibodies (6D7 and 3F3) and the HRP conjugated detector antibody during incubation in the microstrip wells. After washing, the TMB substrate is added. Subsequently the reaction is stopped and the absorbance is read. The developed color is directly proportional to the concentration of the analyte.

3 ASSAY SPECIFICITY

UBC measures defined epitopes on cytokeratin 8 and 18, using the monoclonal antibodies 6D7 and 3F3. No detectable cross reactivity to other tumor associated antigens that may be present in samples exists.

4 SPECIMENS

Voided mid-stream urine (retained in the bladder ≥ 3 h) is recommended. The urine specimens should be centrifuged (1000 x g for 10 minutes) and the supernatant should be diluted 1:10 in UBC Urine Diluent (1x conc.). The diluted urine is stable for maximum 5 days when refrigerated (2-8°C). If delayed analysis, diluted specimens should be frozen in aliquots (<-18°C). Avoid repeated thawing and freezing.
Do not use contaminated urine samples or samples showing gross hematuria.

5 PRECAUTIONS FOR USERS

1. The UBC ELISA is for research use only.
2. Wear protective gloves and protective goggles.
3. Do not use the kit after expiry date.
4. Do not mix reagents from different lots.
5. All specimens should be regarded as contagious and handled and disposed of according to appropriate regulations.
6. Avoid microbiological contamination of reagents.
7. Analysis should be performed according to GLP.
8. The accuracy of the test is related to adherence to the assay procedure and accurate volume pipetting.
9. The Stop Solution contains 0.5 M sulfuric acid, which might cause irritation on skin, and is harmful to eyes. In case of contact flush with plenty of water and seek medical advice.
10. The TMB Substrate might cause irritation on skin and eyes. In case of contact flush with plenty of water and seek medical advice.
11. ProClin 300 (60 ppm) used as preservative in this product might be allergenic. In case of contact flush with plenty of water and seek medical advice.
12. Material Safety Data Sheets are available on request.

CE

RUO in the USA

Revised 10 Dec. 1010 rm (Vers. 1.1)

6 MATERIALS REQUIRED BUT NOT PROVIDED

Microplate reader (wavelength 450 nm).
Microplate shaker (oscillation ~450 rpm).
Microplate wash equipment.
Routine laboratory equipment, e.g. precision pipettes and vortex.

7 DILUTION OF THE UBC ELISA STANDARD

Reconstitute the UBC ELISA Standard with 1.0 ml fresh deionized water.
Let the vial stand for 15 minutes at room temperature (RT).
Add 3.0 ml of UBC Diluent (Standard 0 µg/l) to make a total volume of 4.0 ml.
Mix by vortexing. This is the 15 µg/l Standard.

To prepare respectively Standard concentration dilute according to the table.
Table

Standard (µg/l)	Standard 15 µg/l (µl)	Diluent (std 0 µg/l) (µl)
0	0	1000
1	100	1400
2.5	100	500
5	600	1200
10	600	300
15	1000	0

8 COMPONENTS IN THE UBC ELISA

Materials supplied for 96 determinations.

1. **UBC Coated Microstrips:** 1 plate, 96 dry wells (12x8),
   coated with monoclonal anti-cytokeratin 8/18 antibodies (6D7 and 3F3).
   Packed in aluminum bag with desiccating device. Ready for use.
2. **UBC ELISA HRP Conjugate:** 2 vials, 0.5 ml/vial,
   conjugated antibody in protein stabilized buffer, pH 7.5 (11 x conc.).
   Should be diluted with UBC Diluent (Standard 0 µg/l). Blue colored. Preservative added.
3. **UBC Urine Diluent:** 1 vial, 20 ml,
   sample diluent, protein stabilized buffer, pH 7.5 (2.5 x conc.).
   Should be diluted with 30 ml fresh deionized water. Preservative added.
4. **UBC Diluent (Standard 0 µg/l):** 2 vials, 15 ml/vial,
   standard diluent and standard 0 µg/l, protein stabilized buffer, pH 7.5.
   Preservative added. Ready for use.
5. **UBC ELISA Standard:** 1 vial (4 ml),
   lyophilized cytokeratin material in protein stabilized buffer, pH 7.5. Preservative added.

CE

RUO in the USA

Revised 10 Dec. 1010 rm (Vers. 1.1)

6. **UBC Control (Low, High):** 2 vials control (1 ml/vial),
   lyophilized cytokeratin material in protein stabilized buffer, pH 7.5. Yellow colored. Preservative added.
7. **Wash Tablet:** 1 package, 1 tablet/package.
   The tablet should be dissolved in 500 ml fresh deionized water.
8. **TMB Substrate:** 1 vial, 22 ml.
   Protect from light and keep lid tightly closed. Do not sample more than what is needed for the analysis. Ready for use.
9. **Stop Solution:** 1 vial, 12 ml,
   0.5 M sulfuric acid. Ready for use.
10. **Sealing Tape:** 1 sheet,
    Sealing tape for microstrips.
11. **UBC ELISA Certificate:** 1 protocol.
    Certificate of lot content.

9 ASSAY PROCEDURE

The assay (see Flow chart) should be performed at room temperature (RT; 22 ± 6°C).

1. Allow all reagents and samples to adjust to RT. Vortex all reagents prior to use.
2. Dilute the UBC Urine Diluent with 30 ml fresh deionized water to 1 x conc.
3. Dilute specimens 1:10 with the 1 x conc. UBC Urine Diluent.
4. Prepare the standards (see above).
5. Dilute the UBC Control (Low, High) with 1.0 ml fresh deionized water. Let control vials stand, mix thoroughly after 10 min. Ready to use 15 min after reconstitution.
6. Based on the number of strips needed, dilute UBC ELISA HRP Conjugate (11 x conc.) with UBC Diluent (Standard 0 µg/l) (5.0 ml/vial). Mix thoroughly.
7. Pipette 100 µl standards, controls or samples per well (duplicates). Start with two empty wells for background absorbance measurement (blank).
8. Add 100 µl diluted HRP Conjugate to each well, except the two empty wells.
   Cover the strips with the supplied Sealing Tape.
   NOTE: Steps 7 and 8 should be performed sequentially without interruption.
9. Incubate for 2 h ± 2 min on shaker (~450 rpm).
10. Prepare the wash solution. Dissolve Wash Tablet in 500 ml fresh deionized water.
11. Aspirate and wash the wells 3 x 0.3 ml with wash solution.
12. Add 200 µl TMB Substrate to each well, including the two empty wells. Incubate in darkness for 15 ± 1 min.
13. Add 100 µl Stop Solution to each well. Agitate on shaker 1 min (~450 rpm).
14. Read the absorbance at 450 nm, within 30 min after addition of the Stop Solution.
15. Calculate the cytokeratin 8 and 18 concentration (µg/l) of the samples.
    NOTE: Multiply with the dilution factor. Samples (in 1:10 dilution) showing concentrations >15 µg/l value should be suitably diluted with UBC Urine Diluent before repeated analysis.

CE

RUO in the USA

Revised 10 Dec. 1010 rm (Vers. 1.1)

Flow chart

Prepare standards and HRP Conjugate.
Pipette 100 µl standards/controls/samples per well (duplicates).
Add 100 µl HRP Conjugate per well.
2 h incubation on shaker at RT.
Aspirate and wash.
Add 200 µl TMB. Incubate in darkness 15 min.
Add 100 µl Stop Solution, shake 1 min. Read absorbance at 450 nm.

10 PROCESSING OF RESULTS

Manual calculation or by using a computer software for handling ELISA-type data (curve fitting - Spline smoothed). For generation of valid data, ensure that included controls are within range.

Manual processing of results:
Correct each absorbance value by subtracting the background absorbance (blank).
Estimate the mean value for each duplicate.
Construct a standard curve by plotting the mean absorbance value for each standard (y-axis) against the corresponding concentration (x-axis).
Determine the concentrations of the samples against the standard curve.

11 REAGENT STORAGE

The kit should be stored at 2-8°C. Do not freeze!
Store reagents in their original containers if not used at once.
Reseal the Microstrip bag, including the desiccating device, if not all strips are used at once.
The wash solution is stable for 4 weeks when stored at 2-8°C.
The diluted UBC ELISA HRP Conjugate is stable for 4 weeks when stored at 2-8°C.
The reconstituted and diluted standard is stable for 4 weeks when stored at 2-8°C.
The reconstituted controls are stable for 4 weeks when stored at 2-8°C.
The diluted UBC Urine Diluent (1 x conc.) is stable for 4 weeks when stored at 2-8°C.

12 REFERENCES

1. Stigbrand, T. et al. Epitope specificity of 30 monoclonal antibodies against cytokeratin antigens: The ISOBM TD5-1 Workshop. Tumor Biol 1998; 19:132-152.
2. Sánchez-Carbayo, M. et al. Comparative predictive values of urinary cytology, urinary bladder cancer antigen, Cyfra 21-1 and NMP22 for evaluating symptomatic patients at risk for bladder cancer. J Urol 2001; 165:1462-1467.
3. Giannopoulos, A. et al. Comparative evaluation of the diagnostic performance of the BTA stat test, NMP22 and urinary bladder cancer antigen for primary and recurrent bladder tumors. J Urol 2001; 166:470-475.