PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

Anti-p53

PURE	RUO	REF	IQP-171P	100 tests
FITC	RUO	REF	IQP-171F	100 tests

RUO ***For Research Use Only***

Description

Clone BP53.12

Isotype Murine IgG2a

Specificity Clone BP53-12, produces mouse IgG2a immunoglobulins recognizes both wild and mutant forms of 53 kD protein, identified as p53 suppressor gene.

Antigen distribution

p53 is a tumor suppressor gene encoding a nuclear phosphoprotein that plays an important role in the control of normal cell proliferation. p53 is expressed in peripheral blood and bone marrow cells of patents with some hematological malignance. p53 protein quantitation is of value to ascertain malignancy and provides additional parameter suitable for evaluation of residual disease and for the monitoring of therapy and might also be one of the methods to indicate early relapse [1].

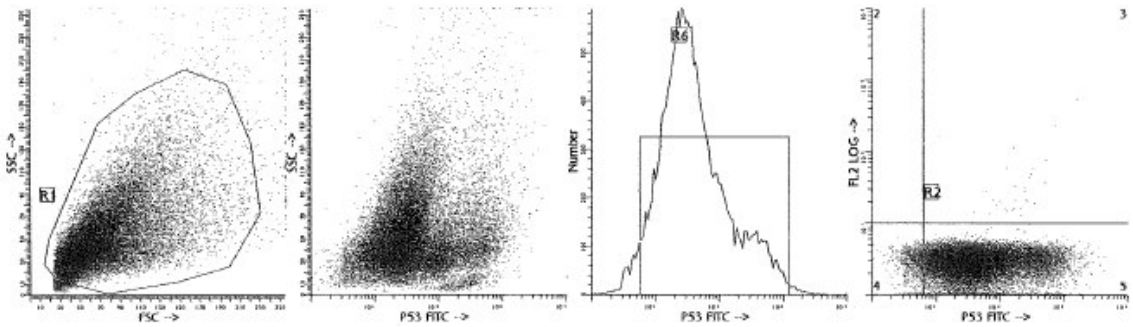
Summary p53 binds to a DNA consensus sequence, the p53 response element, and it regulates normal cell growth cycle events by activating transcription of genes, involved either in progression through the cycle, or causing arrest in the G1 phase when the genome is damaged. In most transformed and tumor cells the concentration of p53 is increased 5-1000 fold over the minute concentrations in normal cells, principally due to the increased half-life (4h) compared to that of the wild-type (20 min).

Applications Monoclonal antibodies to p53, clone BP53.12, can be applied in flow cytometry for analysis of blood and bone marrow and in immunohistochemistry using paraffin tissue sections.

Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10^6 leukocytes for singles and 20 µl/10^6 leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

Representative Data

Clone BP53-12 (anti-p53) monoclonal antibody was analyzed by flow cytometry using Raji cells. Direct staining was performed using 10 µl of anti-p53 FITC and 100 µl cell suspension.
Note: Detection of intracellular antigens needs an adjusted protocol for immuno-staining.



Limitations

1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC and CyQ. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.
2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion. IQ Products has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.
3. Reagents can be used in different combinations, therefore laboratories need to become familiar performance characteristics of each antibody in relation with the combined markers in normal and abnormal samples.
4. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Reagents and materials required but not supplied

1. Flow cytometer
2. Flow cytometry disposable 12 x 75-mm capped polystyrene test tubes
3. Micropipette with disposable tips
4. Vortex mixer
5. Centrifuge
6. IQ Lyse - erythrocyte lysing solution (IQP-199)
7. IQ Starfiqs – fixation and permeabilization solution (IQP-200)
8. PBS (phosphate-buffered saline)
9. 1% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)

Immunofluorescence staining and lysing protocol

Protocol for immuno-fluorescence staining of intracellular antigens

IQ Starfiqs™ is a fixation and permeabilization solution intended for preparation of blood leukocytes before flow cytometry analysis of intracellular antigens. **IQ Starfiqs™** is a **ready to use** product, composed of two reagents used sequentially. The composition of both reagents is adjusted to ensure an optimum performance in flow cytometry analysis. Both reagents should be stored at 4 – 8 °C till the expiration period as indicated.

For optimal intracellular immunostaining and lysing of erythrocytes, **IQ Starfiqs™** should be used following the complete procedure as indicated below (see protocol). **IQ Starfiqs™** enables the detection of intracellular antigens such as CyCD3, CyCD22, TdT and MPO (myeloperoxidase). Results of analysis of blood samples for intracellular detection of MPO are shown below.
In addition, the application of **IQ Starfiqs™** allows the simultaneous detection of cell surface antigens (see extended protocol **IQ Starfiqs™**). It is important to use both reagents and not to mix with other products.
IQ Starfiqs™ is provided as a ready to use product, to minimize hands on time and the easy handling of samples.

Protocol IQ Starfiqs™ (staining of intracellular antigens)

- Add 100 µl EDTA treated whole blood (bone marrow sample, mononuclear cell suspension) to a reagent tube.
- Add 100 µl **IQ Starfiqs™** fixation reagent (Reagent F).
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 100 µl of **IQ Starfiqs™** permeabilization reagent (Reagent P).
- Add 10 µl of IQ Products antibody conjugate for single reagent, or 20 µl of antibody conjugate for dual reagent.
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline.

Extended Protocol IQ Starfiqs™ (staining of cell surface antigens and intracellular antigens)

- Add antibody conjugate to a reagent tube: 10 µl of antibody conjugate for single reagent directed against a cell surface antigen.
- Add 100 µl of EDTA- or Heparin-treated whole blood and mix well.
- Incubate for 15 minutes at room temperature in the dark.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove the supernatant.
- Add 100 µl **IQ Starfiqs™** fixation reagent (Reagent F).
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove the supernatant and resuspend the cell pellet in 100 µl of **IQ Starfiqs™** permeabilization reagent (Reagent P).
- Add 10 µl of IQ Products antibody conjugate for single reagent directed against an intracellular antigen.
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline.

Handling and Storage

Antibodies are supplied either as 100 tests per vial (1 ml) for singles or 50 tests per vial (1 ml) for dual and triple combinations. They are supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 7.3, 0.2% BSA, 0.09% sodiumazide (NaN_3). Store the vials at 2-8 °C. Monoclonal antibodies should be protected from prolonged exposure to light. Reagents are stable for the period shown on the vial label when stored properly.

Warranty Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product.

Characterization

To ensure consistently high-quality reagents, each batch of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

Warning All products contain sodiumazide. This chemical is poisonous and hazardous. Handling should be done by trained staff only.

References

1. Konikova, E., et al., 1999,Flow cytometry of p53 protein expression in some hematological malignancies, Neoplasma, 46(6): 367-376
2. Vogelstein and Kinzler, 1992. Cell, 70: 523-526
3. Hollstein et al. (1991) Science, 253: 49-53
4. Lane, D.P., 1992. Nature, 358: 15-16
5. Donehower et al., 1993. Biochemic. Biophys. Acta. 1155: 181-182

Explanation of used symbols

	Consult instructions for use
REF	Catalogue number
	Sufficient for
IVD	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
RUO	For Research Use Only
LOT	Batch code
	Use by yyyy-mm-dd
	Manufacturer
EC REP	Authorized Representative in the European Community
CE	Conformité Européenne (European Conformity)

		Label - tandem	Ex -max (nm)	Em -max (nm)
P	PURE	purified material	-	-
F	FITC	FITC	488	519
R	R-PE	PE	488, 532	578
C	CyQ	PE-Cy5.18	488, 532	667
A	APC		595, 633, 635, 647	660
PC	PerCP		488, 532	678
PCC	PerCP-Cy5.5		488, 532	695

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