**Specificity analysis of Estrogen Receptor antibody clones using a novel high density protein microarray**

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**Introduction and Aims**

The assessment of estrogen receptor alpha (ER) status by immunohistochemistry (IHC) remains vitally important in directing the treatment of patients with breast cancer. Positivity rates can vary between laboratories due to many factors such as population demography and testing protocol sensitivity. Despite this variation there is no recognized gold standard ER IHC assay. The ASCO/CAP guidelines for IHC testing of ER identifies a number of antibody clones that have well-established specificity and sensitivity demonstrating good correlation with patient outcomes (figure 1).

The aim of this study was to use a novel high density protein microarray to assess the specificity of three commercially available ER alpha antibody clones. Material and Methods

Blinded samples of ER alpha antibody clones - 6F11, SP1 and EP1 were assessed for specificity using a high density protein microarray “chip” developed by OriGene Technologies Inc. (Ma D, et al. 2012). The chip was an Oncyte Avid nitrocellulose coated slide containing 10,000 unique protein targets that had been printed onto the slide. The proteins had been expressed in human HEK293T cells transiently transfected with ORF cDNA expression clones and obtained through gentle cell lysis. Microarray chips were probed with the ER primary antibodies to determine if they would react specifically with the intended target protein. Stained microarrays were scanned and the data analyzed.

Results from the chip analyses were verified by Western Blot of the overexpressed recombinant proteins that were identified as being immunoreactive on the chip with the relevant ER alpha antibody.

**Materials and Methods**

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**Results**

**Protein microarray analysis**

The results in figure 2 show that:

- Clone 6F11 recognized two over expressed proteins on the OriGene protein lysate microarray (figure 2A). Estrogen receptor alpha over-expression lysate (ESR1) showed the strongest positive signal. CCDC144NL was also significantly higher than the background signal.

- Clone SP1 recognized four over-expressed proteins on the OriGene protein lysate microarray chip (figure 2B). EDARADD (transcript variant-B) showed the strongest positive signal. ESR1 over-expression lysate showed the second strongest positive signal. EDARADD (transcript variant-A) and MFGE8 were also significantly higher than the background signal.

- Clone EP1 recognized five over-expressed proteins on the OriGene protein lysate microarray chip (figure 2C). ESR1 over-expression lysate showed the strongest positive signal. CCDC144NL was also significantly higher than the background signal.

**Western blot analysis**

Verification of the chip results by Western blot (figure 3) showed that:

- Clone 6F11 recognized only ESR1 but not CDC144NL (figure 3A). This data confirmed that clone 6F11 is specific for Estrogen receptor alpha by using OriGene’s 10K chip test.

- Clone SP1 recognized recognized ESR1, however, it may also cross-react with EDARADD (transcript variant-B) and EDARADD (transcript variant-A) (figure 3B). This data confirmed that clone SP1 is not specific for Estrogen receptor alpha by using OriGene’s 10K chip test.

- Clone EP1 recognized ESR1 and also recognized CTYorH2, MR1, AOC3 and UBA1 (figure 3C). This data confirmed that clone EP1 is not specific for Estrogen receptor alpha by using OriGene’s 10K chip test.

**Conclusion**

- 6F11 was shown to be specific for estrogen receptor alpha. SP1 was shown to recognize estrogen receptor alpha and two variants of the protein EDARRAD. EP1 recognized estrogen receptor alpha and four other proteins.

- The results of the OriGene 10K protein chip assay suggest that 6F11 is a more specific antibody for Estrogen Receptor alpha than SP1 and EP1.

**Reference**