



Appendix A: VENTANA HER2 Dual ISH DNA Probe Cocktail Scoring Form

1. Case ID / Patient ID: \_\_\_\_\_

2. Is this case enumerable? 2a.  Yes-Proceed to #3  
2b.  No-Skip #3. Proceed to #4

3. Is tumor heterogeneity present? 3a.  Yes, Skip #4. Proceed to #5.  
3b.  No, Skip #4. Proceed to #5.

4. This case is not enumerable because mark ALL that apply):

4a.  There was no tissue left on the ISH stained slide

4b.  There was no invasive carcinoma in tissue on the ISH stained slide

4c.  Nuclear Morphology is unacceptable; unable to distinguish tissue structural elements of normal cells from those of target carcinoma cells.

4d.  Background unacceptable, interferes with scoring ISH stained slide  
HER2 Chr 17

4e.  Internal Control signal not detectable  
HER2 Chr 17

4f.  Weak/absent ISH staining in target cells, unable to score  
HER2 Chr 17

4g.  Other (specify): \_\_\_\_\_

5. Enumerate Target Area 1: Count HER2 signal and Chr17 signal in each of 20 nuclei. Add the HER2 signal counts. Add the Chr 17 counts. Create the gene status ratio by dividing the TOTAL HER2 signal count by the TOTAL Chr 17 signal count. Round to .1 decimal place. Document whether clusters of signals were counted.

SIGNAL COUNT TARGET AREA 1 – 20 nuclei																						5w	5x Comments
5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	5k	5l	5m	5n	5o	5p	5q	5r	5s	5t	5u	5v		
01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	TOTAL	RATIO	Clusters Present?	
HER2																						<input type="checkbox"/> Yes <input type="checkbox"/> No	
Chr17																						<input type="checkbox"/> Yes <input type="checkbox"/> No	

6. Result from 20 nuclei: 6a.  Non- amplified: HER2/Chr17 < 2.0 or 6b.  Amplified: HER2/Chr17 ≥ 2.0  
If the HER2/Chr17 ratio falls between 1.8 and 2.2 then 20 additional nuclei should be enumerated.

7. Enumerate Target Area 2: Count HER2 signal and Chr17 signal in each of 20 nuclei. Add the HER2 signal counts. Add the Chr 17 counts. Document whether clusters of signals were counted.

SIGNAL COUNT TARGET AREA 2 – 2 <sup>nd</sup> 20 nuclei (if target area 1 ratio is 1.8 – 2.2)																						7v	7w Comments
7a	7b	7c	7d	7e	7f	7g	7h	7i	7j	7k	7l	7m	7n	7o	7p	7q	7r	7s	7t	7u			
01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	TOTAL	Clusters Present?		
HER2																						<input type="checkbox"/> Yes <input type="checkbox"/> No	
Chr17																						<input type="checkbox"/> Yes <input type="checkbox"/> No	

8. Result from all 40 nuclei:

8a. Target Area 1 HER2 Total \_\_\_\_\_ + Target Area 2 HER2 Total \_\_\_\_\_ = Total HER2 count \_\_\_\_\_  
(from box 5u) (from box 7u)

8b. Target Area 1 Chr17 Total \_\_\_\_\_ + Target Area 2 Chr17 Total \_\_\_\_\_ = Total Chr17 count \_\_\_\_\_  
(from box 5u) (from box 7u)

8c. Ratio: Total HER2 / Total Chr17 \_\_\_\_\_

8d. Final Result from 40 nuclei:  Non- amplified: HER2/Chr17 < 2.0 or  Amplified: HER2/Chr ≥ 2.0

Scored by: \_\_\_\_\_

Date: \_\_\_\_\_