APPENDIX

Harris's haematoxylin and eosin procedure

Solution		
1%acid alcohol		
Hydrochloric acid	1	ml
70% ethyl alcohol	99	ml
Ammonia water		
28% ammonium hydroxide	2	ml
Distilled water	800	ml
Saturated litium carbonate		
Lithium carbonate	1.54	gm
Distilled water	100	ml
Eosin phloxine solution		
Eosin stock solution		
Eosin Y, water soluble	1	gm
Distilled water	100	ml
Phloxine stock solution		
Phloxine B	1	gm
Distilled water	100	ml
Eosin-Phloxine working solution		
Eosin stock solution	100	ml
Phloxine stock solution	10	ml
95% ethyl alcohol	780	ml
Glacial acetic acid	4	ml

*The solution is good for approximately 1 week

Harris's haematoxylin

Hematoxylin	5	gm
100% ethyl alcohol	50	ml
Potassium or ammonium, alum	100	gm
Distilled water	1000	ml
Mercuric oxide, red	2.5	gm

Completely dissolve the alum in the distilled water with the aid of heat and a magnetic stirrer. Shake to dissolve the haematoxylin in the alcohol, at room temperature. Remove the alum and distilled water from the heat. Slowly combine the two solutions. Return combined solutions to the heat. Bring to a boil as rapidly as possible, approximately 1 minute or less. Remove from the heat and slowly add the mercuric oxide. If the mercuric oxide is added too rapidly, the reaction will cause the solution to boil up and out of the flask. Return the solution to the heat unit it becomes a dark purple, remove it from the heat, and plunge it into a sink of cold water to cool. The solution is ready for use. Add 20 ml of glacial acetic acid to intensify the nuclear stain. Always filter before each use.

Procedure

1. Deparaffinized sections and dehydrate in graded alcohol and hydrate to distilled water.
2. Stained in freshly filtered Harris's haematoxylin for 6 minutes.
3. Washed in running tap water for 5 minutes.
4. Differentiated in 1% acid alcohol, 1 to 2 dips.
5. Washed briefly in running tap water.
6. Placed in ammonia water until sections are bright blue.
7. Washed throughly in running tap water for 10 minutes.
8. Placed in 80% ethyl alcohol for 1 to 2 minutes.
9. Counterstained in eosin-phloxine solution for 2 minutes.
10. Dehydrate and clear through 2 changes each of 95% ethyl alcohol, absolute ethyl alcohol, and xylene, 2 minutes each.
11. Mount with mounting medium.

Results

Nuclear	blue
Cytoplasm	pink to red
Most other tissue structure	pink to red

Immunohistochemistry technique

Solution

1. Monoclonal Mouse Anti-human p53 protein. Clone DO-7 at concentration 1:2000
2. Normal horse serum
3. Biotinylated anti-mouse IgG
4. Avidin-Biotin complex

Reagent A	1 µl
Reagent B	1 µl
PBS	298 µl

5. Peroxidase substrate kit DAB SK-1400 Vector

   5.1 To 5.0 ml of distilled water, add 2 drops of buffer and mix well.

   5.2 Add 4 drops of DAB stock solution and mix well.

   5.3 Add 2 drops of H_2O_2 and mix well.

6. TE- buffer (pH 9.0)

10 mM Tris	3.633 g
1 mM EDTA	1.116 g
Distilled water	3,000 ml

Procedure

Tissue sections of the tumor will be deparaffinized, dehydrated in graded alcohols ending in distilled water. The pressure cooker will be used for antigen retrieval. Section will be boiled in TE-buffer (10 mM Tris, 1 mM EDTA, pH 9.0) for 7 minutes. After cooling by running tap water

for a few minutes, the blockage of endogenous peroxidase will be performed in 3% hydrogenperoxide (H_2O_2) for 30 minutes at room temperature. Then, the sections will be incubated in 3% normal horse serum for 20 minutes to prevent non-specific binding. Consequently, monoclonal mouse anti-human p53 protein (DAKO) at 1:2000 dilution in 3% normal horse serum will be applied to the sections and leave at room temperature for 1 hour. After washing with phosphate buffer saline (PBS), the sections will be incubated with biotinylated horse anti-mouse immunoglobulin (1:100) for 30 minutes at room temperature. The sections will then be incubated in Avidin-Biotin Complex (ABC kit, Vector Laboratories vectastain®), diluted in PBS at a concentration of 1:300 for 60 minutes at room temperature. After being rinsed three times in PBS, the sections will be reacted in 3,3'-diaminobenzidine tetrahydrochloride (DAB) for 10 minutes. The section will be stopped by washing the sections in distilled water and running tap water for 5 minutes. For counterstaining, sections will be left in haematoxylin for a 1 minute, and then be washed in running tap water to remove access stain. All sections will be dehydrated in 95%, 100% alcohol and cleared in xylene respectively, and mounted with permount. The sections of colorectal cancer will be used as a positive control. The negative control sections will be processed by the same technique with the primary antibody omitted.

Result

The nuclear staining of p53 protein are colored brown

CIRRICULUM VITAE

Name	Miss Jaruwan Ponjaroen
Date of Birth	December 5, 1978
Place of Birth	Ayutthaya
Education	
May 1996	Certificate of Mathayom 6, ChomsurangUpathum School, Ayutthaya
May 2000	Bachelor of science (Radiology), Faculty of Associated Medical Science, ChiangMai University, ChiangMai